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14. ABSTRACT

During Year 1, progress was made in preparation for the initiation of subject enrollment. The study protocol has been finalized and a comprehensive assessment battery incorporating neurocognitive, objective alertness, and subjective symptom assessments has been assembled and finalized. The testing battery was selected on the basis of sensitivity to sleep-inducing agents and military relevance. Study equipment and supplies have been purchased. Scientific and human use approvals have been solicited. Study documentation has been submitted to the appropriate Institutional Review Boards for approval, and an Investigational New Drug application (IND) has been filed with the Food and Drug Administration. An IND number has been assigned which will allow for interstate shipment of study drug. In accordance with relevant federal regulations, the study has been registered with clinicaltrials.gov. Key study personnel have been hired and trained in preparation for upcoming enrollment. As a result of the progress made during Year 1, subject recruitment and enrollment will begin during the early months of Year 2.

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September 30, 2010

Effect of a Hypocretin/Orexin Antagonist on Neurocognitive Performance
USAMRMC Grant W81XWH-09-2-0080
Thomas Neylan, M.D., Principal Investigator

INTRODUCTION

An integrated translational study will be conducted to examine the effect of a novel hypocretin/orexin antagonist, almorexant (ALM), to a standard hypnotic, zolpidem (ZOL), and placebo (PBO) on neurocognitive performance at peak concentration post dosing. The human study component (Task 1; responsible individual: Thomas Neylan, M.D.) will establish whether ALM is superior to ZOL in relation to neurocognitive side effects. It is hypothesized that healthy human subjects receiving zolpidem 10mg will show greater impairment in neurocognitive performance compared to subjects receiving 100mg or 200mg doses of almorexant or placebo. Study subjects (n=200) will receive a randomly assigned, one-time dose of study drug in an inpatient hospital setting. A battery of neurocognitive, objective alertness, and subjective symptom assessments will be administered prior to and following dosing. Assessments to be administered were selected based upon their demonstrated sensitivity to sleep-inducing agents and their military relevance. The animal study component (Tasks 2 – 5; responsible individual: Thomas Kilduff, Ph.D.) will compare the neural circuitry that underlies the activity of the abovementioned compounds, their effects on sleep and performance, and the effects of these compounds on biomarkers associated with normal sleep.

BODY

Progress associated with each task outlined in the approved Statement of Work is listed below:

Task 1: *Test the hypothesis that healthy human subjects receiving ZOL 10mg will show greater impairment in neurocognitive performance compared to subjects receiving PBO or the 2 doses (100mg, 200mg) of ALM.*

According to the approved Statement of Work, the subtasks listed below were projected for completion during Year 1 in association with Task 1:

Subtask #1: Write Protocol

The study protocol has been finalized and approved by the study team. The battery of neurocognitive and objective alertness assessments within the protocol has been finalized and will test functioning in the following domains:

- Sustained Attention/Reaction Time: Psychomotor Vigilance Test, Continuous Performance Test II
- Working/Verbal Memory: Restricted Reminding Task (short term cued and uncued explicit short and long-term memory), Paired-Associates Learning Task, Wechsler

Memory Scale (learning and cued recall), Digit Span Task, Wechsler Adult Intelligence Scale – IV (working memory, attention)

- Executive Function: Tower Test from Delis-Kaplan Executive Function Systems, Stroop Color-Word Test, Continuous Performance Test II

- Visual-Motor Coordination: Grooved Pegboard Test

- Objective Alertness: Maintenance of Wakefulness Tests, Stanford Sleepiness Scale

The assessments above were selected based on their demonstrated sensitivity to sleep-inducing agents as well as their application to militarily relevant aspects of neurocognitive functioning. Additional detail related to the assessment battery can be found in the attached study protocol (Appendix 1).

Subtask #2: Obtain Scientific and Human Use Approvals

Approval must be received from the appropriate Institutional Review Boards (IRBs) and the Food and Drug Administration (FDA) prior to the initiation of enrollment. Progress related to each type of approval is listed below:

- IRB Approval: At the conclusion of Year 1, all study documentation had been provided to the following entities for review and approval: the University of California, San Francisco Committee on Human Research, the U.S. Army Medical Research and Materiel Command Human Research Protection Office, and the Department of Veterans Affairs Medical Center Research and Development Committee. Final approval is expected to be received during the early months of Year 2.

- Investigational New Drug Application (IND) – At the conclusion of Year 1, an IND application was filed with the FDA in order to obtain approval to receive study drug from Actelion Pharmaceuticals. An IND number has been assigned for the investigational product and the FDA has provided authorization for the shipment of study drug.

Subtask #3: Purchase Study Related Equipment/Supplies

The equipment listed below has been purchased in preparation for the initiation of enrollment in early Year 2. Standard Operating Procedures (SOPs) have been written which relate to the study equipment, and the equipment has been tested and piloted in preparation for use during the study.

- Sleep Equipment: Embla Titanium ambulatory recorders have been purchased for the overnight polysomnography recordings and the Maintenance of Wakefulness Tests.

- Actigraphs: Wrist-worn actigraphs have been purchased from Ambulatory Monitoring, Inc. for use during the one-week sleep/wake monitoring period of the study.

- Psychomotor Vigilance Tests (PVT-192): PVTs were purchased from Ambulatory Monitoring, Inc. for use in the neurocognitive testing battery.

- Neurocognitive Testing Supplies: Testing kits, score sheets, and manuals have been purchased for the all necessary neurocognitive tests.

- Computers and Computer Supplies: Dell Precision workstations, monitors, and computer supplies have been purchased for use by study personnel.

- Study Drug Supply: Approvals from the FDA have been provided (see Appendix 3) and arrangements have been made with Actelion Pharmaceuticals, Ltd. for the shipment of study drug. Study drug is scheduled to arrive on site during Q1 of Year 2.

Subtask #4: Hire & Train Laboratory Personnel

Key personnel hired during Year 1 and their contributions to the progress of the study are listed below:

- Neuropsychologist: The neuropsychologist finalized and piloted the neurocognitive testing battery and contributed to the development of SOPs related to the neurocognitive tests. The Neuropsychologist also hired and trained the psychological technicians in preparation for screening and enrolling subjects.

- Sleep Technician: The Registered Polysomnographic Technologist (RPSGT) facilitated the purchase and testing of actigraphs, PVT and all sleep equipment. The RPSGT created SOPs for sleep-related tasks to be performed during the study.

- Psychological Technicians: The Psychological Technicians were trained to conduct diagnostic evaluations in subjects to establish subject eligibility. They were also trained to administer the neurocognitive testing battery and they participated in the study pilots.

- Study Coordinator: The Study Coordinator took the lead in the development and management of the clinical study protocol, Informed Consent Form, Case Report Forms and study SOPs. All regulatory submissions with IRBs and the FDA were completed, and arrangements were made with Actelion Pharmaceuticals for study drug shipment. The Study Coordinator also assisted in the hiring and training of study personnel.

- Database Manager: The Database Manager has completed the following tasks during Year 1: creation of a Data Management Plan, design of Case Report Forms, design of a study database, identification of necessary data collection tools and technology platforms for the study, and development of Data Management SOPs.

Other Accomplishments Completed During Year 1:

- Study planning meetings have taken place between key study personnel and the research staff at the University of California, San Francisco (UCSF) Clinical Translational and Sciences Institute (CTSI) Clinical Research Center (CRC), where the inpatient portion of the study will take place.

- Study planning meetings have taken place between key study personnel and the CRC at the San Francisco VA Medical Center, where eligibility screening assessments will be performed.

- Dr. Neylan attended the annual meeting of the Associated Professional Sleep Societies (APSS) and met with collaborators from Actelion Pharmaceuticals Ltd., Walter Reed Army Institute of Research (WRAIR), and SRI International.

- The San Francisco (human study) and SRI International (animal study) teams met monthly via teleconference throughout Year 1 to share progress updates, scientific rationale, and future planning initiatives. The annual investigator/collaborator meeting was hosted by the San Francisco team which was attended by SRI International. Members from each team gave presentations related to research rationale, progress, and future directions. Please refer to the attached meeting agenda (Appendix 4).

Tasks 2 – 5: Please refer to the attached report from Dr. Kilduff (Appendix 2) which details the progress made in reference to the animal studies.

KEY RESEARCH ACCOMPLISHMENTS

Task 1 Accomplishments:

- The clinical study protocol and testing battery have been finalized.
- Study documentation has been submitted to IRBs for review and approval.
- An IND has been filed with the FDA; authorization for study drug shipment has been provided.
- Study personnel has been hired and trained on study protocol and procedures.
- The study team has piloted the assessment battery that is included within the protocol.
- Key study equipment has been purchased, tested, and utilized for training purposes, including sleep recording equipment, actigraphs, and neurocognitive testing supplies.

Tasks 2 – 5 Accomplishments:
Please refer to the attached progress report from Dr. Kilduff (Appendix 2).

REPORTABLE OUTCOMES

Reportable outcomes related to Task 1 will not be available until Year 4. Reportable outcomes related to Tasks 2 – 5 are noted in the attached progress report from Dr. Kilduff (Appendix 2).

CONCLUSION

Preclinical data indicate that animals treated with almorexant are easily aroused from sleep and are free of ataxia and other behavioral impairments. If this observation is confirmed in humans, it would have enormous implications for the management of disturbed sleep in both military and civilian populations. The purpose of this research is to test related hypotheses in both animals and humans. In terms of the human component of the project, steps have been taken during Year 1 to secure the resources and approvals necessary to proceed with enrolling human subjects beginning in Year 2. In terms of the animal component of the project, research has just

commenced during Year 1, but the validity of the abovementioned claims and the mechanisms that may underlie them will be tested further in the upcoming years of the project.

APPENDICES

Appendix 1: Human Study Protocol

Appendix 2: Animal Studies Progress Report

Appendix 3: IND Authorization Letter

Appendix 4: Agenda for Annual Meeting between San Francisco (human site) and SRI International (animal site)

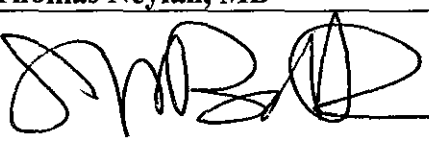
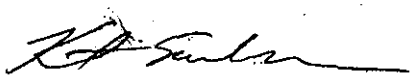
Appendix 1: Human Study Protocol

CLINICAL STUDY PROTOCOL

Title:	A Double-Blind, Placebo-Controlled, Randomized, Parallel-Group Study Comparing the Effect of a Novel Hypocretin/Orexin Antagonist (Almorexant) Versus a Standard Hypnotic (Zolpidem) and Placebo on Neurocognitive Performance
Protocol number:	NEY-1413
Protocol Version/Date:	Final Version 2.0 02 September 2010
Phase:	Investigator-Initiated
Investigational Drug:	Almorexant
Investigator-Sponsor:	Thomas C. Neylan, M.D. Northern California Institute for Research and Education 4150 Clement Street (116P) San Francisco, CA 94121
Medical Monitor:	Steven L. Batki, MD Northern California Institute for Research and Education 4150 Clement Street (116P) San Francisco, CA 94121
Study Sites:	University of California, San Francisco Clinical and Translational Sciences Institute Clinical Research Center 505 Parnassus Avenue San Francisco, CA 94143 San Francisco Department of Veterans Affairs Medical Center 4150 Clement Street San Francisco, CA 94121

This clinical study will be conducted in accordance with Standard Operating Procedures (SOPs), current Good Clinical Practice (GCP) and the provisions of International Conference on Harmonization (ICH) Guidelines

Protocol Approval
NEY-1413
Final Version 2.0 02 September 2010

Investigator-Sponsor		9-17-2010
	Thomas Neylan, MD	**Date**
Medical Monitor		9/17/10
	Steven Batki, MD	**Date**
Sub-Investigator		**9/17/10**
	Kristin Samuelson, Ph.D.	**Date**
Clinical Trial Manager		9/17/10
	Mindy Sivasubramanian, M.S.	**Date**

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ABBREVIATIONS

AE	Adverse Event
AASM	American Academy of Sleep Medicine
BzRAs	Benzodiazepine Receptor Agonists
CRC	Clinical Research Center
CCRC	University of California, San Francisco Clinical Translational and Sciences Institute Inpatient Clinical Research Center
UCSF CHR	University of California, San Francisco Committee on Human Research
CNS	Central Nervous System
CPT	Conners' Continuous Performance Test II
CRF	Case Report Form
DMP	Data Management Plan
DS	Digit Span Subtest of the Wechsler Adult Intelligence Scale Fourth Edition
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders Fourth Edition Text Revision
EEG	Electroencephalogram
FDA	Food and Drug Administration
GABA	Gamma-Aminobutyric Acid
GCP	Good Clinical Practice
GP	Grooved Pegboard Motor Test
ICH	International Conference on Harmonisation
IND	Investigational New Drug Application
IQ	Intelligence Quotient
IRB	Institutional Review Board
MWT	Maintenance of Wakefulness Test
NREM	Non-Rapid Eye Movement
ORP HRPO	Federal Office of Research Protections Human Research Protection Office
P-A	Paired Associates Learning Task
PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index
PVT	Psychomotor Vigilance Test
QC	Quality Control
R&D Committee	Veterans Affairs Research and Development Committee
REM	Rapid Eye Movement

RRT	Restricted Reminding Task
SAE	Serious Adverse Event
SC	Symptom Checklist
SCID	Structured Clinical Interview for DSM-IV TR Axis I Disorders
SFDVAMC	San Francisco Department of Veterans Affairs Medical Center
SSS	Stanford Sleepiness Scale
Stroop	Stroop Color-Word Test
Towers	Tower Test from Delis-Kaplan Executive Function System
USAMRMC	U.S. Army Medical Research Materiel Command
WAIS-IV	Wechsler Adult Intelligence Scale Fourth Edition
WASO	Wake after Sleep Onset
WMS	Wechsler Memory Scale

SYNOPSIS

Protocol Number:	NEY-1413
Study Title:	A Double-Blind, Placebo-Controlled, Randomized, Parallel-Group Study Comparing the Effect of a Novel Hypocretin/Orexin Antagonist (Almorexant) Versus a Standard Hypnotic (Zolpidem) and Placebo on Neurocognitive Performance
Number of Sites:	1
Treatment Duration:	One-time Dose
Study Duration:	10 days, with a follow-up visit within 5 – 12 days of dosing
Study Population:	216 healthy male and female volunteers
Rationale:	In recent years, there has been increased focus on neurocognitive effects of hypnotic medications that adversely affect behavior during unanticipated awakenings during the night. Concerns regarding untoward effects of hypnotics during the sleep period have led to a Food and Drug Administration (FDA) class warning for all hypnotic drugs. These concerns are particularly relevant to the personnel of the military and those in other professions who have an occupational risk of poor sleep and who are expected to perform without impairment upon awakening. Almorexant is a hypocretin/orexin antagonist with a novel mechanism of action that has shown promise as an effective hypnotic. Preclinical data demonstrate that animals treated with almorexant are easily aroused from sleep and behave free of ataxia and other impairment. If this observation is confirmed in humans, it will have substantial implications for the management of disturbed sleep in both military and civilian populations.
Study Objectives:	To compare neurocognitive performance at peak concentration at midpoint during the habitual wake period in subjects randomized to almorexant 100mg, almorexant 200mg, zolpidem 10mg, or placebo.
Study Design:	The study will take place at the San Francisco Department of Veterans Affairs Medical Center (SFDVAMC) and the University of California, San Francisco Clinical Translational and Sciences Institute inpatient Clinical Research Center (CCRC). The study will involve healthy volunteers who are considered normal sleepers per the Research Diagnostic Criteria for Normal Sleepers and who are free of medical disorders and specified psychiatric disorders. After informed consent has been obtained and eligibility has been confirmed, subjects will be scheduled for the 10-day study period.

	During the first seven days of the study period (the sleep/wake monitoring period), subjects will be asked to maintain a sleep diary and wear a wrist activity monitor (actigraph) 24 hours per day. Subjects will be admitted to the CCRC on the eighth day of the study period, two days prior to study drug administration. Subjects' sleep will be monitored with polysomnography (PSG) during each night on the CCRC. Double-blind randomization to one of four groups (almorexant 100mg, almorexant 200mg, zolpidem 10mg, or placebo) will take place after the second night at the CCRC, following the administration of several baseline measures. Study drug will be provided to a nurse on the CCRC by an unblinded research pharmacist. The nurse and all other study personnel will remain blinded when study drug is dispensed to subjects. Following dosing, subjects will be accompanied by study personnel and instructed to remain awake. Neurocognitive, objective alertness, and subjective symptom assessments will be administered for several hours following dosing. Adverse events (AEs) will be assessed at the time of admission to the CCRC and on each day of the subject's stay in the CCRC. Subjects will be debriefed and discharged from CCRC on the morning of the fourth day on the unit. They will be required to return to the CRC at the SFDVAMC within 5 – 12 days of dosing for a safety lab test (liver function).
Inclusion Criteria:	1.) Male and female subjects between the ages of 19 and 39 determined to be physically healthy by physical exam and laboratory assessments; 2.) Habitual wake time between 0600 hr and 0800 hr maintained within the past month; 3.) Habitual bedtime between 2200 hr and 0000 hr maintained within the past month; 4.) Body Mass Index (BMI) >18 and < 28 kg/m^2; 5.) Ability to communicate well with the Investigator and to understand the study requirements.
Exclusion Criteria:	1.) Diagnosis of a sleep disorder within two years of screening or current sleep disturbance as suggested by a global score of > 5 on the Pittsburgh Sleep Quality Index (PSQI); 2.) Current presence of two or more risk categories on the Berlin Questionnaire for sleep apnea and overnight oximetry showing 10 desaturation events per hour or other results which are, in the judgment of the Investigator-Sponsor, suggestive of sleep apnea. 3.) A current or lifetime diagnosis of any psychiatric disorder with psychotic features, major depression, bipolar disorder, panic disorder, obsessive-compulsive disorder, posttraumatic stress disorder, generalized anxiety disorder, dysthymia, or

	agoraphobia without panic disorder, assessed using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) Axis I Disorders (SCID); 4.) A current diagnosis of alcohol or substance abuse or dependence or a history of alcohol or substance abuse or dependence within the past year, assessed using the SCID; 5.) Subjects who are pregnant, lactating, or planning to become pregnant or subjects who are not willing to use an acceptable form of birth control during the study; 6.) Lifetime history of brain injury (including concussions, mild traumatic brain injuries, or loss of consciousness for $\geq$ 10 minutes which resulted in the development of persistent symptoms lasting $\geq$ 1 month), stroke, brain hemorrhage, seizures (not including infantile febrile seizures), epilepsy, or brain infection caused by meningitis, encephalitis, or any other infectious agent. 7.) Systemic illness affecting central nervous system (CNS) function; 8.) Cardiovascular disease (to include but not limited to arrhythmias, valvular heart disease, congestive heart failure, history of myocardial infarction or family history of sudden cardiac death), hypertension, or hypercholesterolemia; 9.) Asthma or other reactive airway diseases; 10.) Hepatic impairment (Child-Pugh A, B, C); 11.) Any other chronic or unstable medical conditions; 12.) Current use of statins, ketoconazole, prescription or over-the-counter medications or herbal supplements containing psychoactive properties or stimulants in the judgment of the Investigator-Sponsor or Medical Monitor; 13.) Treatment with another investigational drug; 14.) Current daily use of any other medication unless specifically approved by the Investigator-Sponsor; 15.) Consumption of grapefruit (including grapefruit juice) or treatment with moderate or strong inhibitors of cytochrome P450 3A4 (CYP3A4) within one week prior to randomization; 16.) Treatment with drugs metabolized by CYP2D6 isoenzyme with a narrow therapeutic index within one week prior to randomization; 17.) Self-reported regular nicotine use within the past 30 days involving > 4 cigarettes per week or > 2 cigarettes per day; 18.) Self-reported consumption of alcohol within the past 30 days of >14 standard drinks per week or $\geq$ 5 standard drinks on any day (men), or > 7 standard drinks per week or $\geq$ 4 standard drinks on any day (women).

	19.) Use of opioids, benzodiazepines, amphetamines, cocaine, cannabis, or any other illicit drugs within 30 days of screening by self report or a urine toxicology screen; 20.) Known liver disease or abnormal liver function tests assessed at the time of screening; 21.) Self-reported regular caffeine use in excess of 200 mg per day on average within six months of screening; 22.) Habitual long sleepers (> 9 hours) or short sleepers (< 5 hours); 23.) Shift work within one month prior to the screening visit or planned shift work during the study; 24.) Subjects who have traveled > 3 time zones within one week prior to the screening visit or any other visit; 25.) Known hypersensitivity or contraindication to any excipients of the drug formulation.
Outcome Measures:	Primary Endpoints: 1.) A comparison between groups on performance on the following neurocognitive measures: Restricted Reminding Task (RRT), Digit Span subtest of the Wechsler Adult Intelligence Scale IV (DS), Grooved Pegboard motor test, Paired-Associates subtest of the Wechsler Memory Scale (P-A), Stroop Color-Word Test (Stroop), Tower Test from Delis-Kaplan Executive Function System (D-KEFS Tower), Psychomotor Vigilance Test (PVT), and Conners' Continuous Performance Test II (CPT). 2.) A comparison between groups on latency to sleep onset measured by Maintenance of Wakefulness Tests (MWT) at 30 minutes and 150 minutes post-dose. 3.) A comparison between groups on low frequency EEG power during artifact free wake time as measured during MWTs. Secondary Endpoints: 1.) A comparison between groups on latency to sleep onset measured by MWTs at 270 and 390 minutes post-dose. 2.) A comparison between groups on Stanford Sleepiness Scale (SSS) scores. Covariates: 1.) Polysomnography (PSG) – Total Sleep Time on the night prior to the day of dosing. 2.) Actigraphy – Average sleep duration.
Statistical Considerations:	It is hypothesized that subjects receiving zolpidem 10mg will show greater impairment in neurocognitive performance compared to subjects receiving placebo, almorexant 100mg, or almorexant

	200mg. This hypothesis will be tested by comparing groups on post-medication performance tests using pre-medication test scores as covariates. Where multiple administrations of a performance test are given either pre-or post-medication, mixed effects models will be used, with the group by time (i.e., pre- vs. post-medication) interaction effect serving as the test of the hypothesis. Where a test is administered only once pre- and post-medication, the statistical test will be a one-way ANCOVA comparing mean scores on the four groups, with the pre-medication test score serving as the covariate. Planned comparisons will be conducted to compare the zolpidem 10mg group with placebo, almorexant 100mg, and almorexant 200mg separately. P-value adjustments will be made for multiple endpoint variables within any given neurocognitive domain using a step-down non-parametric re-sampling-based procedure. Primary analyses will be intent-to-treat, including all subjects randomized regardless or dropout or missing data status. Missing data will be carefully characterized and multiply imputed if necessary.

1. INTRODUCTION

1.1 Background

In recent years, there has been increased focus on cognitive side effects associated with sleep-inducing medications that may contribute to unusual behavior during unexpected awakenings during the night. Concerns regarding these side effects have led to a Food and Drug Administration (FDA) class warning for all sleep-inducing medication. These concerns are particularly important to the military and other professions that have an occupational risk of poor sleep and being unexpectedly awakened with an expectation to perform without impairment.

Almorexant is a hypocretin/orexin antagonist with a novel mechanism of action that has shown promise as an effective hypnotic. Hypocretin/orexin is a neuropeptide system that stimulates arousal and is involved in sleep regulation. Disruption of the hypocretin/orexin system has been shown to result in the sleep disorder narcolepsy in both animals and humans, indicating that this system is part of the intricate sleep/wakefulness regulatory network. Hypocretin receptors are found in many brain regions, although receptor expression is weak in the cortex and high in brain regions associated with arousal state regulation, particularly the histaminergic, serotonergic, noradrenergic and cholinergic wake-promoting systems. Since the hypocretin peptides are excitatory throughout the brain, hypocretin antagonists work by blocking this excitation rather than producing a generalized inhibition. To the contrary, benzodiazepine receptor agonists (BzRAs) such as zolpidem affect gamma-aminobutyric acid ($GABA_A$) receptors which have widespread distribution in the central nervous system (CNS), particularly in the cerebral cortex. BzRAs therefore cause a general inhibition of neural activity (2).

1.1.1 Preclinical Background

Preclinical data demonstrate that almorexant produces a profile that is unique among currently marketed hypnotic medications. For example, preliminary study results in rats treated with one of three doses (10mg/kg, 30mg/kg, and 100mg/kg) of almorexant, zolpidem or placebo in the middle of the dark active period (six hours after lights offset) demonstrated that the 30mg/kg and 100mg/kg doses of almorexant and zolpidem increased non-rapid eye movement (NREM) sleep for several hours after dosing, whereas 10mg/kg of almorexant had a more transient effect. All three doses of almorexant increased rapid eye movement (REM) sleep while REM was suppressed by zolpidem. Consequently, the REM-NREM ratio was unchanged relative to vehicle in animals treated with almorexant, but zolpidem produced a decreased REM-NREM ratio which is characteristic of BzRAs. When cumulative effects were assessed over the entire six-hour post-treatment period, it was evident that almorexant produced a dose-dependent decrease of wake and a dose-dependent increase in both NREM and REM sleep. This profile of a proportional increase of REM and NREM sleep appears to be unique among currently marketed hypnotic medications (3).

Additionally, almorexant appears to have few side-effects on regulated physiological systems. Preliminary studies comparing the effects of varying doses of almorexant,

zolpidem, and placebo on core body temperature in rats revealed that zolpidem-treated animals experienced a significant and prolonged change in core body temperature post-treatment, but there was relatively little change in core body temperature associated with any dose of almorexant (3).

In studies involving somnolent rats treated with almorexant, the rats showed an immediate reversibility of the hypnotic effect with no impairment on motor performance tasks (3). If similar observations are confirmed in humans, there will be enormous implications for the management of disturbed sleep in both military and civilian populations.

1.1.2 Clinical Background

Because hypocretins are implicated in coordinating states of wakeful vigilance, there has been a rapid development of small molecule hypocretin 1 and hypocretin 2 antagonists for possible use in insomnia. At present, there are robust drug discovery programs for hypocretin1/hypocretin 2 antagonists sponsored by Actelion, Glaxo-Smith Kline, Merck, Banyu, Sanofi-Aventis, and Janssen. In 2007, Actelion presented results of a multi-site, double-blind placebo controlled trial in insomnia patients examining the effects of 50mg, 100mg, 200mg, and 400mg doses of almorexant at bedtime. The results showed significant improvement in sleep efficiency and reduced wake after sleep onset (WASO) at doses of 100mg and higher (4). There was no occurrence of cataplexy at any of the dosages used. Almorexant has an elimination half-life of 1.4 hours and effects on sleep electroencephalography (EEG) were absent after 6.5 hours (3).

Almorexant was well-tolerated in studies completed to date, including eighteen Phase I studies in healthy and hepatically impaired subjects, two dose-finding studies in adult and elderly patients with primary insomnia, and one Phase III study in primary insomnia. 439 healthy and hepatically impaired subjects were exposed to at least one dose of almorexant in Phase I studies. 633 subjects with primary insomnia have been exposed to at least one dose of almorexant in completed studies. Maximum exposure was up to 400mg daily for 1 day or up to 200mg for 16 days. 166 patients with primary insomnia received 200mg for at least 14 days, and 176 received 100mg for at least 14 days. The most frequently reported adverse events with almorexant were headache, fatigue, dizziness, and somnolence (40).

1.2 Rationale

At appropriate doses, all currently available FDA-approved prescription sleep-inducing agents induce restorative sleep. However, they also exert substantial performance-impairing effects at peak concentration in multiple domains of neurocognitive function. For example, multiple studies have shown impairment in driving within the six-hour window after ingesting zolpidem (6, 7). Other studies have documented impairment in balance and postural tone within two hours of taking zolpidem (8). Furthermore, there is solid evidence that at peak concentration, currently available sleep-inducing agents significantly impair the ability to consolidate new memories (9-12). This evidence therefore precludes the use of sleep-inducing agents under operational conditions in

which individuals might be called upon to perform without impairment after taking the agent, which is particularly relevant to populations involved in military combat. Further, there is an enormous accumulation of data linking disturbed sleep to a wide range of outcomes including daytime fatigue (13-15), impaired concentration and attention (16-19), increased risk for accidents and injuries (20, 21), worsened quality of life (22), increased aggression (23-26), and increased use of alcohol (27, 28). Several studies have also demonstrated that disturbed sleep is a potent risk factor for later onset development of major depression, panic disorder, alcohol, and substance abuse (27-30). Therefore, an effective treatment for sleep disturbances that can be safely utilized in deployed military personnel in combat operations without performance-impairing effects has the potential for improving the success of combat operations, inoculating soldiers against battlefield stress-related psychiatric illnesses, and preserving the psychological health of the soldiers throughout the full deployment lifecycle. The availability of such a treatment would also have a positive impact on the overall quality of life, physical, and psychological well being of the civilian population.

The study discussed in this protocol will involve a double-blind, placebo-controlled, randomized, parallel-groups study design and will involve a one-time oral administration of one of four dosing options to healthy volunteers: almorexant 100mg, almorexant 200mg, zolpidem 10mg, and placebo. These dosages have demonstrated favorable safety profiles in clinical trials (5). Subjects will be dosed at the average midpoint of the habitual wake period. Neurocognitive performance assessments will be administered at the time of peak plasma concentration. The study will establish whether almorexant is superior to zolpidem and placebo regarding neurocognitive performance at the estimated peak plasma concentration.

2. CLINICAL STUDY OBJECTIVES

2.1 Primary Objectives

Primary endpoints are listed below:

1.) A comparison between groups on performance on the following neurocognitive measures: Restricted Reminding Task (RRT), Digit Span subtest of the Wechsler Adult Intelligence Scale IV (DS), Grooved Pegboard motor test (GP), Paired-Associates subtest of the Wechsler Memory Scale (P-A), Stroop Color-Word Test (Stroop), Tower Test from Delis-Kaplan Executive Function System (D-KEFS Tower), Psychomotor Vigilance Test (PVT), and Conners' Continuous Performance Test II (CPT).
2.) A comparison between groups on latency to sleep onset measured by Maintenance of Wakefulness Tests (MWT) at 30 minutes and 150 minutes post-dose.
3.) A comparison between groups on low frequency EEG power during artifact free wake time as measured during MWTs.

2.2 Secondary Objectives

Secondary endpoints are listed below:

1.) A comparison between dosing groups on latency to sleep onset measured by MWTs at 270 and 390 minutes post-dose.
2.) A comparison between dosing groups on Stanford Sleepiness Scale (SSS) scores.

The following outcomes will be analyzed as covariates:

1.) Polysomnography (PSG) - Total Sleep Time on the night prior to the day of dosing.
2.) Actigraphy – Average sleep duration.

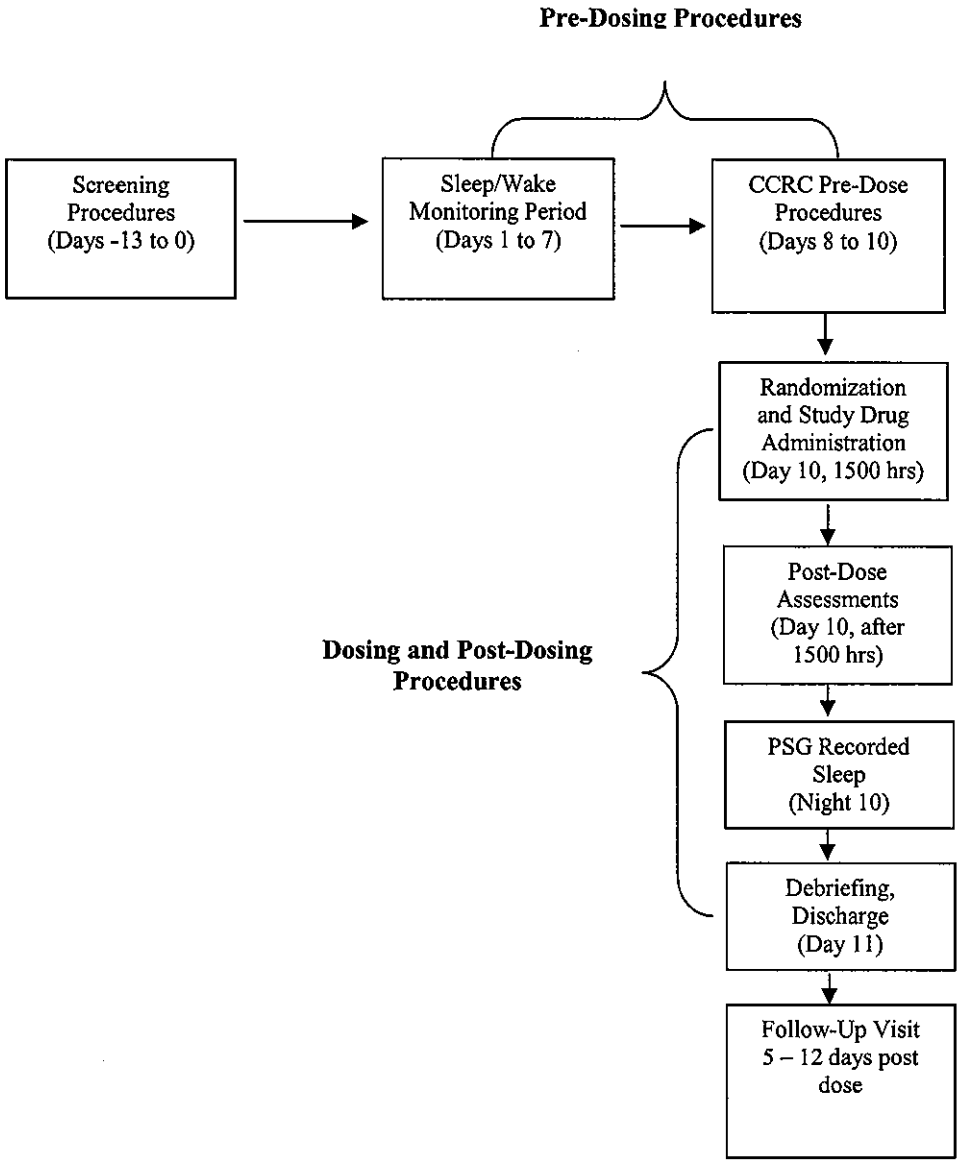
3. STUDY DESIGN

The study will take place at the San Francisco Department of Veterans Affairs Medical Center (SFDVAMC) and the University of California, San Francisco Clinical Translational and Sciences Institute inpatient Clinical Research Center (CCRC). The study will involve healthy volunteers who are considered normal sleepers per the Research Diagnostic Criteria for Normal Sleepers (1) as listed below:

1.) Subject has no complaints of sleep disturbance or daytime symptoms attributable to unsatisfactory sleep.
2.) Subject has a routine sleep/wake schedule characterized by regular bedtimes and rising times.
3.) There is no evidence of a sleep-disruptive medical or mental disorder.
4.) There is no evidence of sleep disruption due to a substance exposure, use, abuse, or withdrawal.
5.) There is no evidence of a primary sleep disorder.

Subjects will also be free of medical disorders and specified psychiatric disorders. After informed consent has been obtained and eligibility has been confirmed, subjects will be instrumented with wrist actigraphs to record their sleep/wake patterns for seven days; subjects will also be asked to complete a sleep diary during this one-week time period. Subjects will be admitted to the CCRC on the day after completion of the one-week sleep/wake monitoring period and two days prior to drug administration. Subjects' sleep will be monitored with PSG during each night at the CCRC, and sleep apnea will be screened for during the first night of PSG. Double-blind randomization to one of four groups (almorexant 100mg, almorexant 200mg, zolpidem 10mg, or placebo) will take place after the second night at the CCRC. An unblinded research pharmacist will provide study drug to a nurse on the CCRC for dispensing. The nurse and all other study personnel will remain blinded when study drug is dispensed to subjects. Following dosing, subjects will be accompanied by study personnel and instructed to remain awake. Neurocognitive, objective alertness, and subjective symptom assessments will be administered at regular intervals for several hours following dosing. Adverse events (AEs) will be assessed at the time of admission to the CCRC and on each day of the subject's stay in the CCRC. Subjects will be debriefed and discharged from the CCRC during the morning of the fourth day on the unit. They will be required to return to the CRC at the SFDVAMC within 5 – 12 days of dosing for a safety lab test (liver function).

3.1 Study Design Schematic



4. SUBJECT SELECTION

Medically healthy men and women ages 19-39 (N = 216) will be recruited from newspaper advertisements, web based postings, websites, and flyers posted in various university and community sites. The age range is restricted to an upper limit of 39 years as a result of research showing a change in middle-aged individuals (defined as 40+ years of age) in terms of total sleep time and other sleep parameters that can affect performance outcomes independent of sleep deprivation and/or drug administration, which could therefore introduce a substantial source of error variance into the study (31). Interested potential subjects will be contacted by the study recruiter. If potential subjects agree, a 15 – 30 minute phone discussion will take place to determine whether they might be a match for the study. If the phone conversation indicates that the potential subjects may be a match for the study and they are still interested, they will be scheduled to meet with the study coordinator or another qualified study team member in person at the SFVAMC for informed consent and further eligibility procedures.

4.1 Subject Inclusion Criteria

Subjects must meet all inclusion criteria in order to be eligible for the study:

1.) Male and female subjects between the ages of 19 and 39 determined to be physically healthy by physical exam and laboratory assessments;
2.) Habitual wake time between 0600 hr and 0800 hr maintained within the past month;
3.) Habitual bedtime between 2200 hr and 0000 hr maintained within the past month;
4.) Body Mass Index (BMI) >18 and < 28 kg/m^2;
5.) Ability to communicate well with the Investigator and to understand the study requirements.

4.2 Subject Exclusion Criteria

Any of the following criteria will exclude the subject from entering the study:

1.) Diagnosis of a sleep disorder within two years of screening or current sleep disturbance as suggested by a global score of > 5 on the Pittsburgh Sleep Quality Index (PSQI) (43);
2.) Current presence of two or more risk categories on the Berlin Questionnaire (42) for sleep apnea and overnight oximetry showing 10 desaturation events per hour or other results which are, in the judgment of the Investigator-Sponsor, suggestive of sleep apnea.
3.) A current or lifetime diagnosis of any psychiatric disorder with psychotic features, major depression, bipolar disorder, panic disorder, obsessive-compulsive disorder, posttraumatic stress disorder, generalized anxiety disorder, dysthymia, or agoraphobia without panic disorder, assessed using the Structured Clinical Interview for DSM-IV TR Axis I Disorders (SCID) (41);

4.) A current diagnosis of alcohol or substance abuse or dependence or a history of alcohol or substance abuse or dependence within the past year, assessed using the SCID (41);
5.) Subjects who are pregnant, lactating, or planning to become pregnant or subjects who are not willing to use an acceptable form of birth control during the study;
6.) Lifetime history of brain injury (including concussions, mild traumatic brain injuries, or loss of consciousness for $\geq$ 10 minutes which resulted in the development of persistent symptoms lasting $\geq$ 1 month), stroke, brain hemorrhage, seizures (not including infantile febrile seizures), epilepsy, or brain infection caused by meningitis, encephalitis, or any other infectious agent.
7.) Systemic illness affecting central nervous system (CNS) function;
8.) Cardiovascular disease (to include but not limited to arrhythmias, valvular heart disease, congestive heart failure, myocardial infarction or family history of sudden cardiac death), hypertension, or hypercholesterolemia;
9.) Asthma or other reactive airway diseases;
10.) Hepatic impairment (Child-Pugh A, B, C);
11.) Any other chronic or unstable medical conditions;
12.) Current use of statins, ketoconazole, prescription or over-the-counter medications or herbal supplements containing psychoactive properties or stimulants in the judgment of the Investigator-Sponsor or Medical Monitor;
13.) Treatment with another investigational drug;
14.) Current daily use of any other medication unless specifically approved by the Investigator-Sponsor;
15.) Consumption of grapefruit (including grapefruit juice) or treatment with moderate or strong inhibitors of cytochrome P450 3A4 (CYP3A4) within one week prior to randomization;
16.) Treatment with drugs metabolized by CYP2D6 isoenzyme with a narrow therapeutic index within one week prior to randomization;
17.) Self-reported regular nicotine use within the past 30 days involving $>$ 4 cigarettes per week or $>$ 2 cigarettes per day;
18.) Self-reported consumption of alcohol within the past 30 days of $>$14 standard drinks per week or $\geq$ 5 standard drinks on any day (men), or $>$ 7 standard drinks per week or $\geq$ 4 standard drinks on any day (women).
19.) Use of opioids, benzodiazepines, amphetamines, cocaine, cannabis, or any other illicit drugs within 30 days of screening by self report or a urine toxicology screen;
20.) Known liver disease or abnormal liver function tests assessed at the time of screening;
21.) Self-reported regular caffeine use in excess of 200 mg per day on average within six months of screening;
22.) Habitual long sleepers ($>$ 9 hours) or short sleepers ($<$ 5 hours);
23.) Shift work within one month prior to the screening visit or planned shift work during the study;
24.) Travel of $>$ 3 time zones within one week prior to the screening visit or any other visit;

25.) Known hypersensitivity or contraindication to any excipients of the drug formulation.

5. STUDY DRUG HANDLING

5.1 Allocation to Dosing Groups

Subjects will be randomly assigned to one of four dosing groups in a 1:1:1:1 ratio: almorexant 100mg, almorexant 200mg, zolpidem 10mg, or placebo. Randomization will be stratified based on gender and caffeine use. Subjects will dose one time on Study Day 10 at 1500 hrs according to their assigned dosing group.

Almorexant (100mg and 200mg) is currently being investigated in a comprehensive Phase III program. Results indicate that almorexant was well-tolerated in the initial Phase III study. Further Phase III studies to evaluate long-term efficacy and safety are in preparation (4).

Zolpidem 10mg is an imidazopyridine class sedative hypnotic which received original United States market approval under the brand name Ambien® in 1992.

5.2 Breaking the Blind

The blind will be maintained through study completion except for cases of breaking the blind due to emergency medical necessity. In situations in which the CCRC nursing staff or other study personnel determines that it might be necessary to break the blind, he/she will be instructed to contact the Investigator-Sponsor or Medical Monitor. If approval is granted by the Investigator-Sponsor or Medical Monitor, the CCRC nurse will be authorized to contact the research pharmacist at the CCRC. The research pharmacist will maintain a master randomization list and he/she or an authorized designee will be available to break the blind if necessary.

5.3 Dosing Adherence/Study Compliance

Since only one dose will be administered to subjects by a nurse at the CCRC, deviations from the scheduled dosing regimen are not anticipated.

During the sleep-wake monitoring period which will take place throughout the week prior to admission to the CCRC, subjects will be required to maintain regular wake times between 0600 hr and 0800 hr and bedtimes between 2200 hr and 0000 hr. Additionally, subjects will be asked to avoid recreational drug use, naps, the consumption of grapefruit or grapefruit juice, alcohol, and/or nicotine. Subjects will also be asked to maintain stable caffeine use and to avoid crossing more than three time zones. Actigraphs will be utilized to monitor the subjects' sleep-wake patterns and will therefore serve as a check for compliance with the prescribed sleep regimen. Subjects will maintain daily sleep diaries during the sleep/wake monitoring period which will capture the following items: lights out and wake clock times, estimated sleep latency, wake time in minutes after sleep

onset, rating of sleep quality on a scale of 1-100, caffeine use, and atypical events. Actigraphy and sleep diary data will be reviewed upon admission to the CCRC to determine compliance with the required sleep/wake schedule. An additional urine toxicology screening will be administered at the time of admission to the CCRC to rule out recent recreational drug use, and females will receive a urine pregnancy test at this time.

5.4 Drug Supplies

5.4.1 Formulation and Packaging

Actelion Pharmaceuticals Ltd. will provide almorexant 100 mg tablets, zolpidem 10 mg capsules, and matching placebo tablets and capsules. A double dummy design will be employed which will result in each subject receiving two tablets and one capsule. Study drug will be provided in bulk and will be shipped directly to the research pharmacy at the CCRC.

5.4.2 Preparing and Dispensing

The research pharmacist in the CCRC will maintain a copy of the randomization schedule. Upon subject randomization, the research pharmacist will dispense the assigned study drug to the nurse who will be administering the drug to the randomized subject.

5.4.3 Drug Administration

After obtaining the appropriate study drug from the research pharmacy, a CCRC nurse will administer the drug to the subject.

5.5 Drug Storage and Accountability

All drug products will be stored at the recommended temperature (room temperature at a maximum of 25°C). Site personnel and study monitors will perform regular checks to document that the study drug is stored appropriately and is within the defined expiration period at all times. A drug accountability log will be completed by the research pharmacist when study drug is received and dispensed to subjects. Any unused drug will be destroyed at the conclusion of the study.

5.6 Concomitant Medications

Medication use will be assessed at screening. Concomitant medications will also be assessed when the subject arrives at the CCRC on Day 8, on each subsequent day in the CCRC (Days 9, 10, and 11), and at follow-up. All concomitant medications will be recorded in the source documents and transcribed onto the Case Report Forms (CRFs).

5.6.1 Disallowed Concomitant Medications and Dietary Restrictions

Use of statins, prescription or over-the-counter medications containing psychoactive properties or stimulants is exclusionary and is also prohibited during the study period. Subjects will be required to maintain stable caffeine consumption of 200 mg per day or less during the study. Alcohol, recreational drug, and nicotine use is prohibited during the 10 day study period. Consumption of grapefruit (including grapefruit juice) or treatment with moderate or strong inhibitors of cytochrome P450 3A4 (CYP3A4) within one week prior to randomization is prohibited.

6. STUDY PROCEDURES

6.1 Pre-Dosing Procedures

Screening (Days – 13 to 0)

The study coordinator or another qualified, trained study team member will obtain informed consent from each potential subject prior to the initiation of eligibility procedures. During the informed consent meeting, the study will be explained and the subject's questions will be answered. Subjects will be allowed to take as much time as they need to make a decision and will be given the option of discussing their decision with their family, friends, or other healthcare providers.

- Physical Exam, Medical History, and Prior/Concomitant Medications Assessment (performed by a nurse practitioner at the SFVAMC CRC).
- Laboratory Analysis of Blood and Urine Samples: A urine sample and approximately 20ccs of blood will be collected for laboratory tests which will include a serum chemistry panel, liver function tests (including albumin), thyroid function tests, prothrombin time, complete blood count and differential, urine toxicology screen, and a urine pregnancy test (in women of childbearing potential).
- Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (SCID [41]), performed by a trained mental health clinician
- Self-report Berlin Questionnaire (42) to determine likelihood of sleep disordered breathing. If subjects have two or more positive scoring categories, they will also be monitored with pulse oximetry.
- Self-report Pittsburgh Sleep Quality Index (PSQI [43])
- Review of Inclusion/Exclusion Criteria

All screening assessments will be performed at the SFDVAMC, including the collection of blood and urine samples and laboratory analysis.

Sleep/Wake Monitoring (Days 1 to 7)

A seven-day sleep/wake baseline monitoring period will be scheduled for subjects who meet all inclusion and exclusion criteria. For female subjects, the baseline monitoring period will be scheduled such that Days 8 - 10 correspond to the follicular phase of the

menstrual cycle. Prior to the start of the baseline week, practice versions of the PVT and RRT will be administered. Additionally, the Vocabulary Subtest of the Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV [48]) will be administered for the purpose of obtaining an IQ measure to ensure that all dosing groups are matched on intelligence. Subjects will be asked to wear wrist actigraphs 24 hours per day on each day of the seven day monitoring period, and they will also be asked to abide by the following instructions:

- Adhere to a consistent wake schedule of 0600 hrs – 0800 hrs and a lights out schedule of 2200 hrs – 0000 hrs.
- Avoid nicotine and recreational drug use.
- Maintain stable caffeine consumption of ≤ 200 mg per day.
- Avoid alcohol use.
- Avoid the consumption of grapefruit or grapefruit juice.
- Avoid travelling > 3 time zones.
- Avoid naps.
- Avoid starting new medications unless they become necessary in the opinion of a physician.
- Use an acceptable form of birth control.

Subjects will maintain daily sleep diaries during the sleep/wake monitoring period which will capture the following data points: lights out and wake clock times, estimated sleep latency, wake time in minutes after sleep onset, rating of sleep quality on a scale of 1-100, caffeine use, and atypical events.

Day 8 (CCRC Admission)

Subjects will enter the CCRC in the evening and a urine toxicology screen will be performed. A urine pregnancy test will be performed for female subjects of childbearing potential. Whether or not female subjects are in the follicular phase of their menstrual cycles will also be assessed at the time of admission. All subjects will be asked to report concomitant medications and AEs dating back to informed consent. Actigraphs will be collected and sleep diary data will be reviewed to determine compliance with the required sleep/wake regimen. Compliance with other study-related instructions will also be assessed at this time. While at the CCRC, subjects will receive a prescribed lights out time which will be consistent with the lights out regimen that was followed during the baseline week. All subjects will be prescribed a 0700hr wake time during their stay at the CCRC.

During the night, subjects will have their sleep monitored with ambulatory PSG. Subjects will also be screened for obstructive sleep apnea which will involve thermistor measurements, pulse oximetry for detection of oxygen desaturation events, and two channels of respiratory effort utilizing strain gauges to measure chest and abdominal movement during breathing. Subjects with an apnea/hypoapnea index ≥ 10 will be excluded from the data analysis.

Day 9

Subjects will be awakened at 0700 hrs and will remain in the CCRC for monitoring. Caffeine consumption should remain consistent with what the subject consumed throughout the sleep/wake monitoring period. Naps will be prohibited. During the evening (prior to lights out), AEs and concomitant medications will be assessed. Subjects will have their sleep monitored with PSG.

Day 10 (Pre-Dose; 0700hrs – 1500hrs)

Subjects will be awakened at 0700 hrs. Caffeine consumption will remain consistent with what the subject consumed throughout the sleep/wake monitoring period. Beginning at 1000 hrs, subjects will be administered a series of baseline (pre-dose) neurocognitive assessments, objective alertness assessments, and subjective assessments. All assessments will be administered by qualified, trained, research technicians. Assessments to be administered are described below:

Stanford Sleepiness Scale: Subjects will be asked to rate themselves along a 7-point scale ranging from 1 (fully alert) to 7 (extremely sleepy). This scale will be administered just prior to each administration of the MWT. Administration time is less than 5 minutes.

Maintenance of Wakefulness Test: Subjects will be placed in a dimly lit room where they will sit comfortably and receive instruction to keep their eyes open and attempt to remain awake while being monitored via standard MWT EEG leads. If the subject falls asleep, he/she will be awakened after three epochs of sleep as determined by EEG trace. Administration time is 20 minutes.

Psychomotor Vigilance Test: Subjects will be required to press a button each time a target is presented. Administration time is 10 minutes.

Restricted Reminding Task – Form 1: Each subject will be read a list of 20 words (nouns from a single semantic category) and asked to repeat as many of the words as possible from the list. On each subsequent trial, subjects will be reminded of the words not previously recalled and asked again to repeat as many of the words as possible from the entire list. The administration continues until the subject repeats the entire list twice in a row, or until a total of eight trials have been administered. Administration time is approximately 10 – 15 minutes.

Paired-Associates Learning Task – Form A: Subjects will be read 10 pairs of words. They will then be read, in a different order, the first word from each pair for which they are to recall the associated second word. The list will be presented and followed by recall two more times (with pairs in a different order each time). This administration will test immediate recall, during which errors are corrected. Administration time is approximately 5 – 7 minutes.

Continuous Performance Test II: Subjects will be required to press the space bar or click the mouse button when any letter except for the target letter "X" appears. Administration time is 15 minutes.

Symptom Checklist: Subjects will be asked if they are experiencing specific symptoms commonly associated with hypnotics. If they endorse any of the symptoms on the checklist, they will be asked whether the symptoms are mild, moderate, or severe. Administration time is approximately 5 minutes.

Vital signs (sitting blood pressure and heart rate) will be obtained each time the Symptom Checklist is administered throughout Day 10. Staff will also query for AEs at this time point.

6.2 Study Dosing

Day 10 (Dosing and Post-Dose, 1500hrs - 2200hrs)

Subjects will dose at 1500 hrs. Shortly after dosing, a PVT administration will take place. MWTs (preceded by the SSS each time) will be administered at 1530 hrs, 1730 hrs, 1930 hrs, and 2130 hrs.

Based on the literature (3), it is estimated that almorexant will reach peak blood concentration between 1600 hrs and 1800 hrs. Around this timeframe, subjects will be administered the PVT, CPT, and SC, in addition to the MWT and SSS. The following neurocognitive and assessments will also be administered during this timeframe:

Paired-Associates Learning Task – Forms A and B: During the timeframe of 1600 hrs – 1800 hrs, Form A (given prior to dosing) will be re-administered to subjects to test delayed recall. At a later time point within the time window of 1600 hrs – 1800 hrs, Form B will be administered to test immediate recall. During the administration of delayed recall trials, errors will not be corrected.

Restricted Reminding Task – Forms 3 and 4: Lists of 20 words different from the list read to subjects prior to dosing will be read to subjects who will be required to repeat as many of the words as possible from the list. Form 3 will be administered in the timeframe between 1600 hrs and 1800 hrs, and Form 4 will be administered during the evening. The same administration rules used during pre-dosing will apply.

Grooved Pegboard Test: The test consists of 25 holes with randomly positioned slots. Pegs with a key along one side must be rotated to match the hole before they can be inserted and subjects must place the pegs in the holes as quickly as possible. Administration time is approximately 10 minutes.

Stroop Color-Word Test: Subjects will be given three sheets of paper, one at a time. The Word page consists of the words "red," "green," and "blue" printed randomly in rows in black ink. Subjects will be asked to read as many words as they can out loud in a 45

second time period. The Color page consists of 100 items, all written as "XXXX," printed in either green, red, or blue ink. Subjects will be asked to name as many colors as they can out loud in a 45 second time period. The Color-Word page consists of the words from the Word page printed in the colors from the Color page. The words and the colors they are printed in do not match one another. Subjects will be asked to name as many colors as they can in a 45 second time period. Total administration time is approximately 10 minutes.

Tower Test from Delis-Kaplan Executive Function System: Subjects will be asked to complete problem-solving tasks which will involve moving disks on pegs to match an arrangement shown to them in a picture. Administration time is approximately 20 minutes.

Digit Span: Subjects will be read a sequence of digits and asked to repeat the digits in the same sequence. For the second portion of the test, subjects will be read a sequence of digits and asked to repeat the digits in reverse order. For the third portion of the test, subjects will be read a sequence of digits and asked to repeat the digits in order from the lowest number to the highest. Administration time is approximately 6 minutes.

After the time window of 1600 hrs - 1800 hrs, subjects will receive additional administrations of the PVT, SC, and RRT (third form). Two more MWT administrations will also take place. The final assessment will begin at 2130 hrs.

Study personnel will remain with the subjects throughout testing and subjects will be kept awake until all assessments have been completed.

Night 10 (Post-Dose)

AEs will be assessed prior to the prescribed lights out time. Subjects will engage in undisturbed, PSG recorded sleep.

Day 11 (Discharge)

Upon awakening at 0700 hrs, subjects will have all electrodes removed and will be debriefed prior to being discharged from the CCRC. AEs will be assessed prior to discharge.

Safety Follow-Up

Within 5 – 12 days of dosing with study drug, subjects will be required to have a blood draw performed for a liver function test. This procedure will be performed at the SFDVAMC. Approximately 5ccs of blood will be drawn and analyzed at the SFDVAMC laboratory. The occurrence of AEs and concomitant medications since the day of discharge will be assessed.

7. STUDY OUTCOMES AND SAFETY ASSESSMENTS

7.1 Study Outcome Assessment Measures

A description of the measures which will be utilized for the outcome analyses is provided below:

Psychomotor Vigilance Test: The PVT is a widely used instrument that measures sustained attention and reaction time (49). Extensive work with this measure has demonstrated that the PVT is not affected by practice effects and is a highly sensitive measure of the effects of disrupted circadian rhythms from shift work (17) and chronic sleep deprivation (18, 19). PVT-192® devices will be utilized for this study. The PVT has a random inter-stimulus interval of 2-10 seconds and can be collected over a 10 minute period. The main measure will be performance lapses (reaction time > 500 ms) per 10 minute period. Secondary measures will include total time of lapses, frequency of false responses, frequency of non-responses, durations of the 10% fastest and 10% slowest responses, and performance decrement across time on the task.

Stanford Sleepiness Scale: The SSS is a subjective measure of sleepiness in which subjects rate themselves along a 7-point scale ranging from 1 (fully alert) to 7 (extremely sleepy) (50). Subjective sleepiness ratings will be collected in order to verify the sedative effects of zolpidem and the two doses of almorexant.

Maintenance of Wakefulness Test: The MWT is widely used to demonstrate significant pre and post treatment differences in excessive sleepiness. Sleep onset is defined as the first occurrence of > 15 seconds of cumulative sleep in a 30 second epoch. Latency to the first 30 seconds of sleep will be scored online by the attending sleep technologist. The subject will be awakened within 90 seconds of falling asleep.

Restricted Reminding Task: The RRT assesses verbal memory, specifically by measuring both cued and uncued explicit short and long term lexical memory. The test has a demonstrated sensitivity to zolpidem (12, 46). Multiple forms will be available in order to facilitate multiple administrations of the immediate recall version of the task. This assessment will be scored on the basis of the number of correctly recalled words.

Grooved Pegboard Test: A measure of manipulative dexterity, this test requires complex visual-motor coordination (51).

Paired-Associates Learning Task: This associative learning sub-test of the Wechsler Memory Scale tests the ability to learn and recall pairs of words, some of which are related (e.g., north/south) and others which are unrelated (e.g., eagle/jury) (47). Immediate and delayed recall trials will be scored for the number of correctly recalled pairs.

Continuous Performance Test II: The CPT assesses attention and working memory as well as executive function (44). Specifically, the CPT measures response inhibition via commissions (an aspect of executive function) and sustained attention via omissions.

There is evidence in the literature which suggests that continuous performance tasks are sensitive to sleep-inducing agents (34). Scores will be based on response time and errors, inclusive of omissions and commissions.

Stroop Color-Word Test: The Stroop is a widely used putative measure of executive function that measures response inhibition (35). The Color-Word score will be computed, which measures the subject's ability to inhibit or override the tendency to produce the more automatic or dominant response (i.e., to name the color word rather than the color).

Tower Test from Delis-Kaplan Executive Function System: D-KEFS Tower is typically used for the assessment of executive function, specifically to detect deficits in planning, decision making, and problem solving (45). Literature provides evidence of a link between performance on towers tasks and sleep (32).

Digit Span: Digit Span is a subtest of the WAIS-IV which measures attention and working memory and has been found to be sensitive to sleep-inducing agents (36, 48).

The following measures will serve as covariates:

Actigraphy: The primary actigraph measures are habitual sleep onset and offset times and the range of variability around these data points. The wrist actigraph provides continuous activity data using a battery-operated wristwatch-sized microprocessor that senses motion with an accelerometer. Subjects can also indicate lights on, lights off, and other salient events by pressing an event marker on the actigraphs. The actigraphs will be initialized with the ActMe program (Ambulatory Monitoring, Inc.) using the PIM sampling mode in one-minute epochs for conventional actigraphic sleep-wake estimation.

Polysomnography: The primary PSG measure is total sleep time on the night prior to the day of dosing and neurocognitive testing. PSG recordings will be obtained with ambulatory PSG and the parameters recorded will follow current guidelines as defined in the AASM Manual for the Scoring of Sleep and Associated Events (37).

The Embla Titanium ambulatory recorders record up to 34 channels. The sampling frequency ranges from 256Hz to 512 Hz. High and low frequency filters will be added while scoring the data manually and in spectral analysis. 60Hz notch filters may be applied to remove electrical noise. Raw files will be kept with only anti-aliasing filters. Spectral analysis will organize sleep epochs by stage and time. Artifacts will be tagged for removal for spectral analysis.

7.2 Safety Assessment Measures

Symptom Checklist: This checklist captures common symptoms experienced by subjects taking hypnotic medications. Reports of symptoms will be collected in order to compare possible drug side effects.

AEs will be assessed on a regular basis throughout the study and at the follow-up visit.

A liver function test will be performed on all subjects within 5 – 12 days of dosing with study drug.

8. ADVERSE EVENT REPORTING

8.1 Adverse Event Definitions

An AE is defined as any untoward medical occurrence that takes place in a clinical study, regardless of the causal relationship of the event with the investigational drug or study treatment(s). Any event occurring after the clinical trial participant has signed the study informed consent documentation should be recorded and reported as an AE.

An AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the investigational product, whether or not considered related to the investigational product. A new condition or the worsening of a pre-existing condition will be considered an AE.

An abnormal test finding will be classified as an AE if one or more of the following criteria are met: a.) the test finding is accompanied by clinical symptoms; b.) the test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention, including significant additional concomitant drug or other therapy; c.) the test finding leads to discontinuation of subject participation in the clinical study; d.) the test finding is considered an AE by the Investigator-Sponsor of the IND application.

For each AE, the date and time of onset, a description of the event, severity, seriousness, action taken, relationship to the study drug, outcome, and date of resolution will be recorded.

A **Serious Adverse Event (SAE)** is defined as an AE that results in any of the following:

- Death
- Life-threatening event – An event in which the subject is at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Requires hospitalization or prolongs existing inpatient hospitalization, not inclusive of a pre-planned elective hospital admission for treatment of a pre-existing condition that has not significantly worsened or a diagnostic procedure.
- Results in persistent or significant disability or incapacity.
- Results in congenital abnormality or birth defect.
- An important medical event occurs which requires medical intervention to prevent any of the above outcomes. Important medical events are those which may not be immediately life-threatening but may jeopardize the subject and may require intervention to prevent one of the serious outcomes listed above.

An **Unexpected Adverse Event** is defined as any AE in which the frequency, specificity, or severity is not consistent with the risk information described in the clinical protocol or elsewhere in the current IND application or Investigator's Brochure.

8.2 Recording Requirements

8.2.1 Eliciting Adverse Event Information

AEs will be assessed when subjects check into the CCRC and again during each evening at the CCRC. Additionally, subjects will complete a Symptom Checklist at various scheduled time points throughout the day of dosing and asked to report the occurrence of any other AEs.

8.2.2 Recording Requirements

All observed or volunteered adverse drug events (serious or non-serious) and abnormal test findings, regardless of treatment group or suspected causal relationship to the investigational drug or study treatment(s) will be recorded in the subjects' case histories. For all AEs, sufficient information will be pursued and/or obtained so as to permit a.) an adequate determination of the outcome of the event; and b.) an assessment of the causal relationship between the AE and the study drug.

AEs or abnormal test findings felt to be associated with the investigational drug or study treatment(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the Investigator-Sponsor.

8.3 Reporting of Adverse Events

8.3.1 Reporting of Adverse Events to the FDA

Written IND Safety Reports

The Investigator-Sponsor will submit a written IND Safety Report to the responsible new drug review division of the FDA for any observed or volunteered AE that is determined to be a.) associated with the investigational drug or study treatment(s); b.) serious; and c.) unexpected. Each IND Safety Report will be prominently labeled, "IND Safety Report."

Written IND Safety Reports will be submitted to the FDA as soon as possible and within 15 calendar days following the Investigator-Sponsor's receipt of the respective AE information. For each written IND Safety Report, the Investigator-Sponsor will identify all previously submitted IND Safety Reports that addressed a similar AE experience and will provide an analysis of the significance of newly reported AE in light of the previous, similar report(s).

Follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the relevant information is available. If the results

of the Investigator-Sponsor's follow-up investigation show that an AE that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Investigator-Sponsor will submit a written IND Safety Report as soon as possible and within 15 calendar days after the determination was made.

In accordance with FDA requirements, annual safety reports will be submitted to the FDA.

Telephoned IND Safety Reports

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Investigator-Sponsor will notify the responsible review division of the FDA by telephone or facsimile transmission of any observed or volunteered AE that is a.) associated with the use of the investigational drug or study treatment(s); b.) fatal or life-threatening; and c.) unexpected.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Investigator-Sponsor's initial receipt of the respective human AE information.

8.3.2 Reporting Adverse Events to the Responsible IRBs

In accordance with applicable IRB policies of the Veterans Affairs Medical Center Research and Development Committee, University of California, San Francisco Committee on Human Research, and the U.S. Army Medical Research and Materiel Command Human Research Protection Office (USAMRMC HRPO), the Investigator-Sponsor will report, to the IRBs, any observed or volunteered AE that is determined to be associated with the investigational drug or study treatment(s), serious, and unexpected. AE reports will be submitted to the IRBs in accordance with the respective IRB procedures.

Applicable AEs will be reported to the IRBs as soon as possible and, in no event, later than 10 calendar days following the Investigator-Sponsor's receipt of the respective information. Follow-up information to reported AEs will be submitted to the IRB as soon as the relevant information is available. If the results of the Investigator-Sponsor's follow-up investigation show that an AE that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting, the Investigator-Sponsor will report the AE to the IRB as soon as possible, but in no event later than 10 calendar days after the determination was made.

In accordance with the USAMRMC HRPO requirements, unanticipated problems involving risk to volunteers or others, serious adverse events related to participation in the study and all subject deaths related to participation in the study should be promptly reported by phone (310-619-2165), by e-mail (hsrrb@amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC HRPO. A complete written report should follow the initial notification. In addition to the methods above, the complete report can be sent to

the USAMRMC, ATTN: MCMR-ZB-P, 504 Scott Street, Fort Detrick, Maryland, 21702-5012.

The Medical Monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event to the USAMRMC HRPO. At a minimum, the Medical Monitor should comment on the outcomes of the event or problem and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The Medical Monitor should also indicate whether he/she concurs with the details of the report provided by the Investigator-Sponsor. Reports for events determined by either the Investigator-Sponsor or Medical Monitor to be possibly or definitely related to participation and reports of events resulting in death will be promptly forwarded to the HRPO.

8.3.3 Reporting of Adverse Events to Actelion Pharmaceuticals

Copies of all periodic safety reports (including draft versions for review) to be submitted to the FDA will be provided to Actelion at least 10 days prior to their submission to the FDA. Copies of any MedWatch forms submitted to the FDA will be provided to Actelion immediately upon submission to the FDA.

All serious adverse events, regardless of causality and expectedness, will be reported to Actelion within 24 hours of the Investigator-Sponsor's knowledge of the event.

8.3.4 Withdrawal of Subjects Due to Adverse Events

Withdrawal of subjects due to an AE can take place at any time during the study at the discretion of the Investigator-Sponsor. Subjects may also choose to discontinue participation at any time.

9. STATISTICAL METHODS/DATA ANALYSIS

9.1 Study Endpoints

9.1.1 Analysis of Primary Endpoints

It is hypothesized that subjects receiving zolpidem 10mg will show greater impairment in neurocognitive performance and objective measures of sleepiness compared to subjects receiving placebo, almorexant 100mg, or almorexant 200mg. This hypothesis will be tested by comparing groups on post-medication performance tests using pre-medication test scores as covariates. When multiple administrations of a performance test are given either pre-or post-medication, mixed effects models will be used, with the group by time (pre- or post-medication) interaction effect serving as the test of the hypothesis. When a test is administered only once pre- and post-medication, the statistical test will be a one-way ANCOVA comparing mean scores on the four groups, with the pre-medication test score serving as the covariate. Covariates in all models will include total sleep time

measured by PSG on the night before testing and average sleep duration measured by actigraphy. Planned comparisons will be conducted to compare the zolpidem 10mg group with placebo, almorexant 100mg, and almorexant 200mg separately. Post-hoc comparisons will be made to compare placebo vs. almorexant 100mg, placebo vs. almorexant 200mg, and almorexant 100mg vs. almorexant 200mg. For post-hoc comparisons, p-value adjustments will be made using a re-sampling procedure as implemented in the SAS "simulate" adjustment option.

Two-tailed significance tests will be conducted at the p = .05 level. P-value adjustments will be made for multiple endpoint variables within each domain of neurocognitive functioning (verbal memory, attention/working memory, motor skills, executive function, and psychomotor vigilance) and objective sleepiness (sleep onset latency and low frequency EEG power in the MWT). The p-value adjustments will be made using a step-down, re-sampling based procedure (38, 39) which takes into account the correlational structure among the multiple variables. Primary analyses will be intent-to-treat analyses based on all participants randomized, regardless of dropout or missing data status. Dropout rate will itself be analyzed as a secondary outcome variable. Missing data will be carefully characterized, and multiple imputation will be used where necessary. The exact form of each mixed model, for example the correlational structure of repeated measures and whether heterogeneous group variances are included, will be made on the basis of best fit according to the Bayesian Information Criterion (BIC) before any hypothesis testing is conducted. Assumptions of the models (e.g., normal distributions of errors and absence of outliers) will be assessed, and any necessary remedies, such as data transformation or the use of robust standard errors, will be implemented before hypothesis tests are conducted.

Any deviations from the statistical plan will be described in the study manuscript.

9.1.2 Analysis of Secondary Endpoints

Secondary endpoints include sleep latency on the MWT measured beyond the presumed drug activity period at 270 and 390 minutes post-dose (i.e., the "hangover effect"), and subjective sleepiness measured by the Stanford Sleepiness Scale. Secondary analyses will be conducted in a parallel fashion to the primary analyses, but with re-sampling based multiple comparison procedures for all significance tests.

9.2 Sample Size Determination

Enrollment is estimated to include up to 216 subjects to obtain 200 evaluable subjects. An equal number of subjects (up to 54) will be randomly assigned to each dosing group (almorexant 100 mg, almorexant 200 mg, zolpidem 10 mg, placebo). Randomization will be stratified on the basis of gender and caffeine use. With a power of 0.80 and an alpha of 0.05, the planned sample size will allow for the detection of effect sizes (Cohens' f) of approximately 0.29. It is estimated that the effect of zolpidem 10 mg versus placebo on the cognitive performance measures will range from f = 0.34 to f = 0.80, based on prior findings. Given the hypothesis that both doses of almorexant will be associated with

significantly less impairment than zolpidem 10mg, it is possible that a range of effect sizes might be found with almorexant. If almorexant is absolutely no different than placebo, the study will be slightly overpowered to demonstrate its superiority over zolpidem. However, if almorexant has a more subtle impairment effect on cognition, intermediate between that seen with zolpidem 10 mg and placebo, it might become necessary to be able to detect somewhat smaller effects. According to guidelines suggested by Cohen (33), an effect size of f = .14 is considered "small" and f = .39 is considered "medium." Thus, the proposed study is well powered to test its main hypotheses.

9.3 Definition of Analysis Populations

Primary analyses will be intent-to-treat analyses based on all participants randomized, regardless of dropout or missing data status. If there are a substantial number of participant dropouts, separate analyses on completers only will be conducted as a sensitivity analysis, but hypothesis tests will be based on the intent-to-treat sample. No subgroup analyses are planned.

9.4 Safety Analysis

Dosing groups will be compared on each symptom included as part of the Symptom Checklist using Fisher's exact tests or Chi-Square approximations, depending on the frequency of each symptom. No p-value adjustments will be made.

10. QUALITY CONTROL (QC) AND QUALITY ASSURANCE

The study will be carried out according to requirements of the FDA and all other applicable agencies in addition to ICH accepted standards of GCP. All study-specific procedures will be performed according to approved written Standard Operating Procedures. Study monitors will be responsible for ensuring adherence to FDA and ICH guidelines. Study Monitors for this study will be provided by an external contract monitoring group. Regular monitoring of study data and files at the clinical study sites will be performed as defined in the study-specific monitoring plan. Additionally, an authorized representative from the Investigator-Sponsor study team will perform an annual review of study files and training files to ensure adherence to GCP guidelines and study-specific standard operating procedures. Data collected during the study will be subjected to a thorough quality control review by the lead data managers prior to the statistical analysis. Specific requirements related to the data management QC of the study data will be detailed in the Data Management Plan. AE data will be reviewed on an ongoing basis with the Investigator-Sponsor.

11. DATA HANDLING, RECORD KEEPING, AND CONFIDENTIALITY

11.1 Data Recording/Case Report Forms (CRFs)

A CRF will be completed for each subject enrolled into the clinical study. The Investigator-Sponsor will review each completed CRF book and will complete the Investigator Statement. Completion of the Investigator Statement CRF confirms the Investigator-Sponsor's responsibility for ensuring that all data and corrections on the CRF are complete, accurate, and authentic.

Source documents will consist of laboratory and medical history records, screening instruments, actigraphy data, sleep diaries, PSG data, neurocognitive assessments, and subjective symptom measures including the Symptom Checklist, the Stanford Sleepiness Scale, and AE and concomitant medication disclosures. All necessary information from the source documents will be recorded on the CRFs. Where appropriate, certain data files will be merged with the study database electronically. Data recorded on the CRFs will be identical to the data recorded on the source documents. Queries will be issued to address all discrepancies noted within the study data. Any changes made to the study data as the result of a resolved query will be documented in the audit trail within the study database. Specific procedures related to the handling of blank, discrepant, or otherwise spurious data will be detailed in the Data Management Plan. When all data have been entered, validated and queries resolved, the database will be locked.

11.2 Record Maintenance and Retention

The Investigator-Sponsor will maintain records in accordance with GCP guidelines and all applicable regulations and policies, to include:

- FDA correspondence related to the IND and clinical protocol, including copies of submitted Safety Reports and Annual Reports
- IRB correspondence (including approval notifications) related to the clinical protocol, including copies of AE reports and annual or interim reports
- Current and past versions of the IRB-approved clinical protocol and corresponding IRB-approved consent form(s) and, if applicable, subject recruitment advertisements
- Signed FDA Form 1572 Statements of Investigator
- Financial disclosure information
- Curriculum vitae for the Investigator-Sponsor and all clinical protocol sub-investigators and study personnel
- Certificates of required training for Investigator-Sponsor, all sub-investigators, and other relevant study team members
- Listing of printed names/signatures of Investigator-Sponsor and listed sub-investigators
- Normal values for laboratory ranges
- Laboratory certification information
- Instructions for on-site preparation and handling of the investigational drug, other study treatments, and study materials
- Standard procedures for decoding and breaking the study blind
- Master randomization list
- Signed informed consent forms

- Completed Case Report Forms, signed and dated by the Investigator-Sponsor
- Source Documents
- Monitoring visit reports
- Copies of Investigator-Sponsor correspondence to sub-investigators, including notifications of safety information
- Subject screening and enrollment logs (a listing of all volunteers who signed informed consent)
- Subject identification code list
- Investigational drug dispensing and accountability records, including documentation of drug disposal
- Final clinical study report

The Investigator-Sponsor will retain the specified records and reports for a minimum of two years after the marketing application is approved for the investigational drug. If a marketing application is not submitted or approved for the investigational drug, records will be retained until 2 years after investigations under the IND have been discontinued and the FDA so notified.

11.3 Confidentiality

Participation in research will involve a loss of privacy, but information about subjects will be handled as confidentially as possible. Medical records will be created at UCSF and SFVAMC because of subjects' participation in this study. Information related to informed consent and screening test results will be included in the medical records, as well as information pertaining to vital signs, adverse events, and concomitant medications assessed during the hospital portion of the study. Therefore, other doctors may become aware of the individual's study participation. Hospital regulations require that all health care providers treat information in medical records confidentially. At the time of consent, subjects will be asked to sign forms to authorize the release of their personal health information for research purposes.

If it is suspected that the subject is in danger of harming him/herself or someone else, or if child abuse or neglect or elder abuse has occurred, appropriate authorities will be notified as required by law. It is also possible that subjects' research records could be subpoenaed by a court.

If information from this study is published or presented at scientific meetings, subjects' names and other personal information will not be used.

All study data will all be coded with a code number unique to the study. Only study personnel, with the permission of the Investigator-Sponsor, will have access to the key with the name and ID codes. The subject identification code list will be stored electronically in a password-protected, restricted access folder on a secured study server in order to maintain confidentiality. The only individuals receiving access to the code list will be the team member responsible for maintaining the list and a back-up.

The clinical interviews performed at screening will be audio recorded and will be used only by research personnel in order to calibrate the clinicians' ratings on the standardized interview format. The recordings will be labeled with a unique code number and retained in a secure location (digital recordings will be encryped, passcode protected, and stored and accessed via the secure VA server). Recordings will be retained until the conclusion of the study; at that point, they will be erased. Subjects will be informed that their screening clinical interviews will be audio recorded for the purpose of allowing the research team to ensure consistency across all clinical interviews. They will be informed that the recordings will be maintained under secure conditions at all times and identified only by the unique Subject ID number. Subjects will also be informed that the recordings will be deleted after the conclusion of the study.

The Maintenance of Wakefulness Tests performed on Day 10 will be video recorded and will be used only by research personnel for the purpose of confirming subjects' ability to remain awake during the testing process. The recordings will be labeled with a unique code number and retained in a secure location (digital recordings will be encryped, passcode protected, and stored and accessed via the secure VA server). Recordings will be retained until the conclusion of the study; at that point, they will be erased. Subjects will be informed that their Maintenance of Wakefulness Tests on Day 10 will be video recorded for the purpose of allowing the research team to confirm their ability to remain awake during testing. They will be informed that the recordings will be maintained under secure conditions at all times and identified only by the unique Subject ID number. Subjects will also be informed that the recordings will be deleted after the conclusion of the study.

Organizations that may look at and/or copy subjects' medical records for research, quality assurance, and data analysis include representatives from the following:

- UCSF CHR
- FDA
- USAMRMC
- Actelion Pharmaceuticals, Ltd.

12. ETHICS

12.1 Institutional Review Board (IRB) approval

Prior to initiating the study, the Investigator-Sponsor will obtain approval in writing from all required IRBs. Specifically, approval must be obtained from the UCSF Committee on Human Research, the Veterans Affairs Research and Development Committee, and the U.S. Army Medical Research and Materiel Command Office of Research Protections Human Research Protection Office.

Any amendments to the protocol or changes to the informed consent document mustbe approved by all IRBs prior to the implementation of those changes. The only circumstance in which a modification to the current IRB-approved clinical

protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the Investigator-Sponsor will promptly notify the IRBs of the modification.

The IRBs will be promptly notified of any deviation to the protocol that may have an effect on the safety of the subjects and the integrity of the study. This notification will occur as soon as the deviation is identified. All deviations will also be reported in the continuing review report and final study report.

A copy of the approved continuing review report and the local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available.

In the event that the IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of an Investigator-Sponsor's decision to modify the previously accepted clinical protocol, the Investigator-Sponsor will submit a protocol amendment (prior to the implementation of the changes) to the IND describing any change to the protocol that would significantly affect the safety of subjects, the scope of the investigation, or the scientific quality of the study.

Records of IRB approval and other related correspondence will be maintained in the regulatory files for the study and will be subject to periodic audits and reviews by study monitors. Periodic status reports will be submitted to the IRB as required, and AEs/serious AEs will be reported to each IRB per their specific reporting requirements.

12.2 Ethical and Scientific Conduct of the Clinical Study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol, ICH Guidelines on GCP, and relevant policies, requirements, and regulations of the FDA, UCSF CHR, the VA R&D Committee, the USAMRMC ORP HRPO, and all other applicable state and federal agencies. All procedures described in this protocol will be performed according to approved written SOPs unless otherwise stated.

12.3 Subject Informed Consent

The Investigator-Sponsor will make certain that an appropriate informed consent process is in place to ensure that potential research subjects are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The Investigator-Sponsor, or a staff member designated by the Investigator-Sponsor, will obtain the written, signed informed consent of each subject prior to performing any study-specific procedures. The date and time that

the subject signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The Investigator-Sponsor will retain the original copy of the signed informed consent form and a copy will be provided to the subject.

The Investigator-Sponsor will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the Investigator-Sponsor will obtain the informed consent of enrolled subjects for continued participation in the clinical study

13. EARLY DISCONTINUATION CRITERIA

A subject may withdraw or be withdrawn from the study for the following reasons:

1.) Subject withdrew consent
2.) Investigator judgment
3.) Protocol violation(s)
4.) Non-compliance
5.) Adverse Event
6.) Pregnancy
7.) Other

If subjects withdraw consent prior to admission to the CCRC, they will be asked to return to the SFDVAMC for an early discontinuation visit which will entail an assessment of AEs and concomitant medications, a debriefing, and the return of study-related equipment.

If it becomes necessary to stop parts or all of the clinical study for the safety of the subjects, Actelion, the IRBs, and the FDA will be notified promptly of the discontinuation of the entire clinical study. Respective protocol modifications will be submitted prospectively to the IRB and to the FDA for discontinuation of parts of the clinical study. All sub-investigators will be notified of any necessary discontinuations.

Subjects participating in the study at the time of the discontinuation of a portion or all of the study will be promptly notified and advised of the impact of the discontinuation upon their study schedules. If a portion of the study is discontinued, subjects will be provided with revised informed consent documentation which will need to be signed prior to their continued enrollment in the study.

14. RISKS AND BENEFITS

Study-related risks and associated measures to minimize the risks are listed below:

Study Drug Related Side Effects

Some subjects might experience side effects associated with the study drugs. The list of possible side effects presented below is based on side effects that have been observed in clinical trials involving Almorexant and Zolpidem. Participants in these clinical studies took many different dosages of these drugs ranging from 1mg to 1000mg. Subjects will be told to discuss any side effects with study personnel as they occur. The nursing staff at the CCRC and study personnel will also closely monitor subjects on the day of dosing with study drug. All subjects will have a liver function test performed within 5 – 14 days of dosing with study drug.

Risks and side effects related to taking Almorexant include those which are:

Likely (occurring in greater than 20% of people)

- Drowsiness

Less Likely (occurring in less than or equal to 20% of people)

- Fatigue
- Headache
- Dizziness
- Nausea

Rare but Serious

- Heart rate abnormality (less than 1%)

Risks and side effects related to taking Zolpidem include those which are:

Less Likely (occurring in less than or equal to 20% of people)

- Dizziness
- Drowsiness
- Headache
- Diarrhea
- Fatigue

Rare but Serious

- Heart rate abnormality (less than 1%)
- Severe allergic reaction (less than 1%)

Blood Drawing (Venipuncture)

The risks of drawing blood include temporary discomfort from the needle stick, bruising, and rarely, infection. The amount of blood collected to determine eligibility is approximately 20 ccs or 4 teaspoons. Only a qualified phlebotomist will draw blood following standard SFVAMC lab procedures.

Clinical Interview & Questionnaires

The interview and questionnaires may be distressing to some participants. Subjects will be told that they are free to decline to answer any questions or to stop the interviews at any time.The interviewer will be available to immediately assist with any problems that arise in the interview and will make a referral if required.

Audio Recording – Clinical Interview

The clinical interviews will be audio taped. The audio taping may make subjects somewhat more uncomfortable than they would be without the taping. Research personnel will use the recordings in order to calibrate the clinicians' ratings on the standardized interview format. The audio recordings will be maintained under secured conditions (i.e., the recordings will be encrypted, protected with a pass code, and stored and accessed via a secure server), identified only by a unique ID number, and retained until the conclusion of the study, at which point they will be erased/deleted.

Actigraphy
There is no risk of injury from wearing the actigraph. Subjects might find it annoying to have to wear the actigraph 24 hours per day during the baseline week. Subjects will be told that they can discuss any difficulties with this procedure with study personnel at any time. Subjects will also be able to decline to participate in this procedure at any time.

Polysomnography
There is no risk of injury from any of the recording devices, but subjects might experience slight discomfort from the attached electrodes and tape. High quality hypoallergenic materials will be used to minimize this risk.

Video Recording – Maintenance of Wakefulness
The Maintenance of Wakefulness Tests that will be conducted on Study Day 10 will be videotaped. The video recording may make subjects somewhat more uncomfortable than they would be without the taping. These recordings will only be reviewed by research staff and our consultants for the purpose of confirming subjects' ability to remain awake during the testing. The recordings will be identified by a unique ID number and will be stored under secure conditions (i.e., they will be encrypted, protected with a pass code and stored on a secure server). The recordings will be retained until the conclusion of the study, at which point they will be destroyed.

Maintenance of Wakefulness Tests
There is no risk of injury from taking this test, but subjects might find it annoying or difficult to remain awake while sitting quietly in a comfortable position. Subjects might also become bored while sitting still for the 20 minute duration of the test. Subjects will be able to stop the procedure at any time if they become uncomfortable.

Neurocognitive Assessment Battery
There is no risk of injury from completing the neurocognitive assessment battery, but subjects might become bored, frustrated, or find it difficult to concentrate as you take these tests throughout the day of testing. Subjects will be able to stop the procedures at any time if they become uncomfortable.

Sleepiness
There is a 3 out of 4 chance that subjects will take a sleep aid on Study Day 10 while at the hospital. Therefore, subjects might become sleepy during the study testing procedures, and the study staff will require subjects to remain awake. This might be difficult or frustrating for subjects.

Reproductive Risks
Subjects should not become pregnant or father a baby while participating in this study because the potential effects of the study drugs on an unborn baby are not known at this time. Women should not breastfeed a baby while on this study. Study staff will educate subjects regarding the importance of using appropriate birth control throughout the study.

Unknown Risks
The experimental drugs used in this study may have side effects or discomforts that no one knows about yet. Subjects will be told to discuss any side effects with study personnel as they occur. The nursing staff at the CCRC and study personnel will also closely monitor subjects on the day of dosing with study drug.Subjects will not experience any direct benefits by participating in the study. However, the study is contributing to medical knowledge related to the cognitive effects of sleep aids. Results could have implications for personnel of the military and/or other professions who have an occupational risk of poor sleep.

15. STUDY PERSONNEL

15.1 Investigator-Sponsor

The Investigator-Sponsor will assume overall scientific and administrative leadership for the study. He will be responsible for supervising the study team with regards to the recruitment, diagnostic assessment, and enrollment of subjects and the coordination of all study procedures.

The Investigator-Sponsor will have overall responsibility for the standardization of data collection, data quality control, data analysis, and interpretation. He will have overall responsibility for subject safety, rights, and welfare. He will be an active participant in the preparation of abstracts and manuscripts and will assure the dissemination of study findings in the professional and scientific communities.

15.2 Medical Monitor

The Medical Monitor will provide backup medical coverage which will entail a review of eligibility assessments and safety monitoring (including breaking the study blind if necessary). The Medical Monitor will review all unanticipated problems involving risk to subjects or others, serious adverse events, and all subject deaths associated with the protocol and provide written reports of these events, indicating whether there is agreement with the reports provided by the Investigator-Sponsor.

15.3 Co-Investigators

The Co-Investigators assigned to this study will assist the research team in data collection, data analysis, quality control of study data, data interpretation, and the

preparation of reports. They will provide consultation and oversight to the mental health clinicians and will assist with the determination of eligibility.

15.4 Study Coordinator

The study coordinator will be responsible for the day-to-day activities of the study, including but not limited to the following: obtaining informed consent, subject scheduling, eligibility determination, ensuring the completion of safety reports in a timely manner, case report form completion, ensuring that study team members are properly trained on study procedures, providing oversight to the external study monitors, and providing oversight for data completion, cleaning, analysis, and interpretation. The study coordinator will consult with the project director as necessary for high-level study management and budget oversight.

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Appendix 2: Animal Studies Progress Report

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USAMRAA Grant W81XWH-09-2-0081

TITLE:

EFFECT OF A HYPOCRETIN/OREXIN ANTAGONIST ON NEUROCOGNITIVE PERFORMANCE

PRINCIPAL INVESTIGATOR:

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14. ABSTRACT
During Year 1, progress was made on establishment of the infrastructure and baseline parameters to facilitate the proposed studies. Construction was initiated on a 535 s.f. lab suite within the Animal Facility that will provide an optimal environment for execution of the proposed in vivo studies; we expect to occupy this laboratory next week. An Analytical Neurochemistry Facility was established in LB212 and 4 HPLCs were purchased, installed, calibrated and the minimal detection levels for measurement of several neurotransmitters were determined. Over 25 g of the test compound, the hypocretin receptor antagonist almorexant (ALM), was synthesized, resulting in achievement of the first milestone. The effects of 3 concentrations of ALM on sleep/wake, locomotor activity and body temperature were determined. The behavioral performance studies were initiated in a temporary location. We are now well-positioned to rigorously test the hypothesis in Years 2-4 that ALM produces fewer functional impairments than the benzodiazepine receptor agonist zolpidem (ZOL) because ZOL causes a general inhibition of neural activity whereas ALM specifically disfacilitates wake-promoting systems.

15. SUBJECT TERMS
Sleep, performance, drug, neurotransmitter, hypocretin, orexin, benzodiazepine, zolpidem, neurochemistry, microdialysis

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PROGRESS REPORT
"EFFECT OF A HYPOCRETIN/OREXIN
ANTAGONIST ON NEUROCOGNITIVE PERFORMANCE"
USAMRAA Grant W81XWH-09-2-0081
CDMRP Log No. DR080789P1
Thomas S. Kilduff, Ph.D., Principal Investigator

INTRODUCTION

Almorexant (ALM) is a hypocretin/orexin (Hcrt) receptor antagonist with a novel mechanism of action that has shown promise as an effective hypnotic. Preclinical data demonstrate that animals treated with ALM are easily aroused from sleep and are free of ataxia and other behavioral impairments. If this observation is confirmed in humans, it would have enormous implications for the management of disturbed sleep in both military and civilian populations. The overall hypothesis that underlies this research is that ALM produces fewer functional impairments than the benzodiazepine receptor agonist zolpidem (ZOL) because ZOL causes a general inhibition of neural activity whereas ALM specifically disfacilitates wake-promoting systems. Whereas the human study component will establish if ALM is superior to ZOL in neurocognitive tests, the animal studies will compare the neural circuitry that underlies the activity of these compounds, their effects on sleep and performance, and the effects of these compounds on biomarkers associated with normal sleep.

BODY

As indicated in a 21 Jul 2010 email from the PI, Dr. Thomas Kilduff, to Dr. Kimberly del Carmen, Health Sciences Grants Manager for the Congressionally Directed Medical Research Programs, SRI International has undertaken construction of a new lab for the experiments to be conducted under DR080789P1 (USAMRAA Grant W81XWH-09-2-0080), the "Effect of a Hypocretin/Orexin Antagonist on Neurocognitive Performance". This construction was necessitated because of the type of experiments that were proposed in DR080789P1. Our laboratory for collecting microdialysis samples from rodent brain has been located next to the cage-washing room in the Animal Facility for several years. Although this location was adequate for most microdialysis studies, the location was problematic for the studies proposed in the "Effect of a Hypocretin/Orexin Antagonist on Neurocognitive Performance" since we planned to record sleep/wake in conjunction with obtaining microdialysis samples. This location is a "high traffic" area as cages are moved down the hall and into the washer and up the hall once they have been washed.

Consequently, upon funding of DR080789P1 (USAMRAA Grant W81XWH-09-2-0080), we began planning the construction of a new 535 sq foot laboratory suite in which to conduct the proposed EEG/EMG, behavioral performance and microdialysis studies (see Appendix 1). We anticipated that this would be a minor construction project that would last 6-8 weeks. Unfortunately, the project exceeded a cost threshold which necessitated that the City of Menlo Park to approve the plans, causing the first set of delays. The City assessed our project in the context of the overall facilities in which the laboratory was located and determined that the building as a whole was not compliant with current Americans with Disabilities Act (ADA) regulations. Accordingly, our project triggered a requirement that wheelchair access be provided to the building and that wheelchair-accessible bathrooms be installed. Since the scope of these City-imposed requirements greatly exceeded the scope of our original project, there were further

delays as plans were drawn up and the details of the now-expanded project were negotiated internally. Once construction began, not only was the scope of the construction project larger, but it had to be conducted in a manner that was minimally disruptive to ongoing experiments that were being conducted in the Animal Facility. Thus, construction has been limited to 3 days per week.

Construction on this project began on 19 May 2010, is currently proceeding apace, and is projected to be completed by 03 Aug 2010. We are looking forward to occupying this new laboratory in early August and accelerating progress on the "Effect of a Hypocretin/Orexin Antagonist on Neurocognitive Performance" in this new lab. However, as indicated in the Progress Report below, we have fallen behind on the goals that we established for Year 1 for DR080789P1 (USAMRAA Grant W81XWH-09-2-0080) in the SOW. Although there has been some progress on each of the Tasks, we will achieve fewer of the Milestones in Year 1 than we originally projected.

Task 2. *Test the hypothesis that rodents receiving ZOL will show greater neurocognitive impairment than those receiving ALM or PBO.*
2a. Assessment of Almorexant effects on spatial reference memory in rats (months 1 to 12).
2b. Assessment of Almorexant effects on spatial working memory in rats (months 1 to 12).
2c. Assessment of Almorexant effects on psychomotor vigilance in rats (months 13 to 24).
2d. Synthesis of ALM (months 1-4).

Progress: Task 2d was added to SOW in August 2009 in case problems arose with respect to the donation of ALM that was expected from Actelion Pharmaceuticals Ltd. Logically, however, ALM had to be available before any studies could occur. Thus, Task 2d was the first Task completed. As indicated in the Certificate of Analysis (Appendix 2), **this milestone has been achieved** as 26.05 g of >99% pure almorexant was delivered by the SRI Medicinal Chemistry Laboratory on 31 Mar 2010.

As indicated above, we have been limited in our ability to conduct the studies proposed as Tasks 2a and 2b in the SOW due to the ongoing construction. In May 2010, a small animal experimental room unexpectedly became available for our use on a temporary basis due to the departure of an investigator from SRI. This room was just adequate in size to house the Morris water maze to be used in Aims 2a and 2b. Therefore, we have set up the water maze and video tracking system in this room on a temporary basis and have initiated the studies for Aim 2a and are current collecting data.

Prior to undertaking any of the proposed studies in Tasks 2-5, we had to be certain of the doses of ALM and ZOL to be used for treatment of the animals in these studies. We report here on the results of a study of the effects of ALM and ZOL on sleep and wakefulness undertaken in collaboration with colleagues at F. Hoffman la Roche. Although these experiments were initiated prior to funding of DR080789P1 (USAMRAA Grant W81XWH-09-2-0080), the analysis has only been completed within the past year and the results are directly relevant to the "Effect of a Hypocretin/Orexin Antagonist on Neurocognitive Performance". Male Sprague-Dawley rats (300±25 g) used in this study were from Charles River (Wilmington, MA) and were housed in a temperature-controlled recording room under a 12 h light/12 h dark cycle (lights off at 05:00) with food and water available *ad libitum*. Room temperature (24±2°C), humidity (50±20% relative humidity), and lighting conditions were monitored continuously via computer. Animals were inspected daily in accordance with AAALAC and SRI guidelines.

Experimental design. Eight male Sprague-Dawley rats (300±25 g; Charles River, Wilmington, MA) were implanted with chronic recording devices (F40-EET, Data Sciences Inc., St Paul, MN) for continuous recordings of electroencephalograph (EEG), electromyograph (EMG), core body temperature (T_{core}), and LMA via telemetry as previously described previously (Morairty et al., 2008). Data were recorded using DQ ART 3.1 software (Data Sciences Inc., St Paul, MN). Animals were acclimated to the handling procedures and were given two separate 1 ml doses of vehicle, one 7 d and the other 3 d before the first experimental day. Following completion of data collection, expert scorers determined states of sleep and wakefulness in 10 s epochs by examining the recordings visually using Neuroscore software (Data Sciences Inc., St Paul, MN). Any epochs that contained recording artifacts were tagged and excluded from subsequent analyses. The EEG and EMG data were scored for waking (W), rapid eye movement sleep (REM), and non-REM (NR). T_{core} and LMA (counts per minute) were analyzed as hourly means.

A repeated measures design was employed in which each rat received five separate dosings. The dosing conditions included almorexant at three concentrations (10–100 mg/kg), ZOL (10 mg/kg) and a vehicle control (HPMC). All dosings were administered ip at a volume of 2 ml/kg. A minimum of 3 d elapsed between doses. Dosing occurred during the middle of the rats' normal active period during the start of Zeitgeber hour 19 (ZT19) and was typically completed within 10 min. Animals were continuously recorded for 6 h prior to dosing and for 18 h following dosing.

Data analyses. EEG and EMG data, scored in 10 s epochs as described above, were analyzed as time spent in each state (W, REM, and NR) per hour. Latency to NR onset for each rat was calculated from the time of drug injection to the first six continuous 10 s epochs scored as NR. Latency to REM onset for each rat was calculated from the time of drug injection to the first three continuous 10 s epochs scored as REM. Cumulative time spent in W, NR, and REM, as well as the REM:NR ratios, were calculated for 6 h following drug administration. To determine whether any of the pharmacological treatments affected the consolidation of behavioral states, the duration and number of bouts for each state were calculated in hourly bins. A "bout" consisted of a minimum of two consecutive 10 s epochs of a given state and was terminated by the occurrence of a single epoch of a different state. The EEG spectra during NR sleep were analyzed offline using the fast Fourier transform algorithm in Neuroscore (Data Sciences Inc., St Paul, MN) on all epochs without a visually detectable artifact. EEG delta power (1–4 Hz) within NR (NRD) was then calculated in hourly bins. T_{core} and LMA (counts per minute) were analyzed as mean values per hour (hourly means). Relative T_{core} was calculated as the difference in T_{core} from the 24 h average during the vehicle condition.

Statistics. The records were analyzed in 6 h time blocks (i.e., first half of the dark period, second half of the dark period, first half of the subsequent light period, and second half of the light period) since drug administration occurred 6 h into the recording period. Latency to NR and REM, REM:NR ratios, and cumulative state data were analyzed using one-way repeated-measures analysis of variance (ANOVA); all other data were analyzed using two-way repeated-measures ANOVA. When ANOVA indicated statistical significance, paired two-tailed *t*-tests were performed for post hoc analysis.

Results. Almorexant at 30 and 100 mg/kg reduced NR latency while only the 30 mg/kg concentration decreased latency to REM sleep (Figure 1). ZOL produced the expected decrease in NR latency.

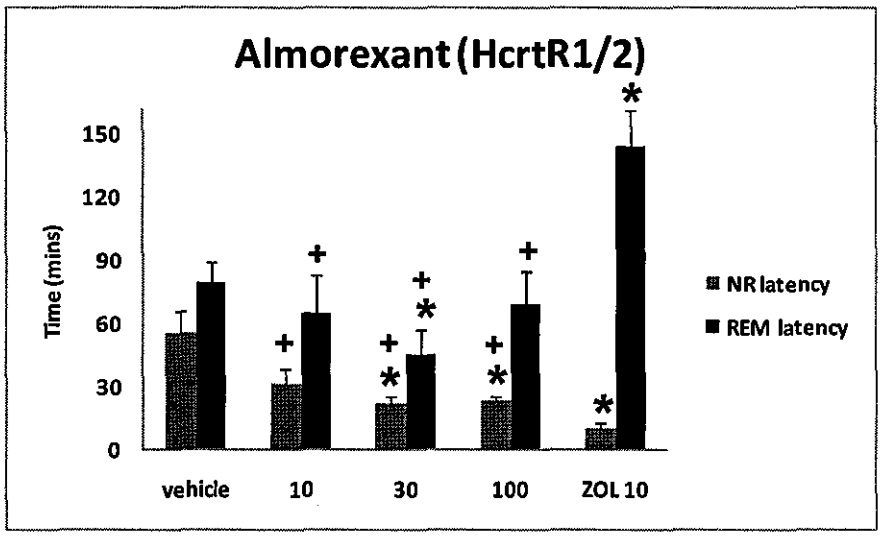


Figure 1. Latency to the onset of NR and REM sleep following administration of almorexant as compared to zolpidem (ZOL). *=significantly different from vehicle (P<0.05); +=significantly different from ZOL (P<0.05) (One-way repeated measures ANOVA followed by paired two-tail *t-tests* test; n=8 per group). Data represent the mean ± SEM.

As illustrated in Figure 2, almorexant (30 and 100 mg/kg) resulted in increased cumulative NR for 2, 4 and 6 h following administration (F=13.010, P<0.0001; F=17.771, P<0.0001; and F=16.179, P<0.0001, respectively). Cumulative REM also increased for the first 2 h following almorexant at 30 mg/kg (F=5.418, P=0.0023) and for the 6 h period following the 100 mg/kg dose (Figure 2; F=8.535, P<0.0001). ZOL increased cumulative NR and decreased cumulative REM. Consequently, whereas ZOL suppressed the REM:NR ratio, ALM did not.

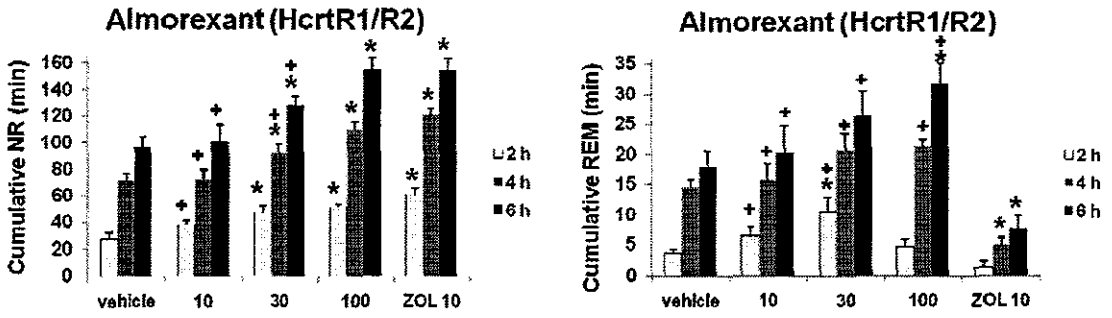


Figure 2. Cumulative time in NR and REM sleep over the first 2, 4 and 6 h following drug administration. **Left:** cumulative time spent in NR sleep following almorexant compared to zolpidem (ZOL). **Right:** cumulative time spent in REM sleep for the same drug treatments. *, significantly different from vehicle; +, significantly different from ZOL.

Although ZOL has significant effects on NRD, ALM did not (Figure 3).

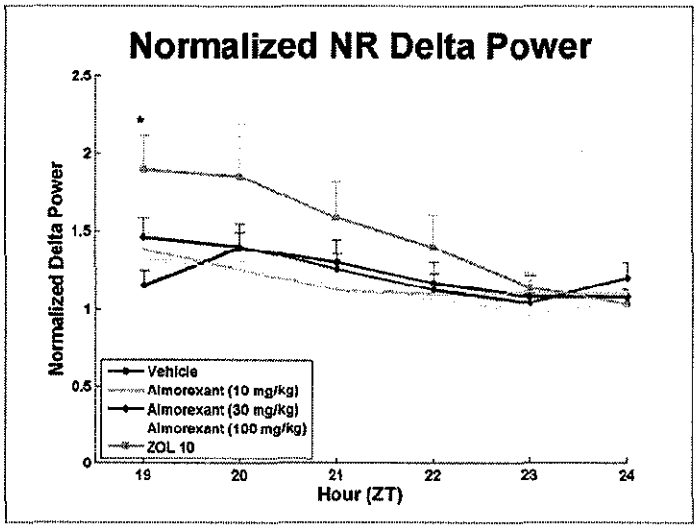


Figure 3. Hourly delta power normalized to the 24 h average vehicle control. 3 concentrations of almorexant vs. ZOL and vehicle.

Both LMA and T_{core} underwent dose-dependent decreases after drug treatment (Figure 4). No differences in LMA during the subsequent light period were found. Condition effects for T_{core} were found. The highest concentration decreased T_{core} across the 6 h period following administration (F=7.315, P=0.00036). ZOL administration resulted in the largest declines in T_{core}, which was followed by a sustained rebound increase in T_{core} during the subsequent light period.

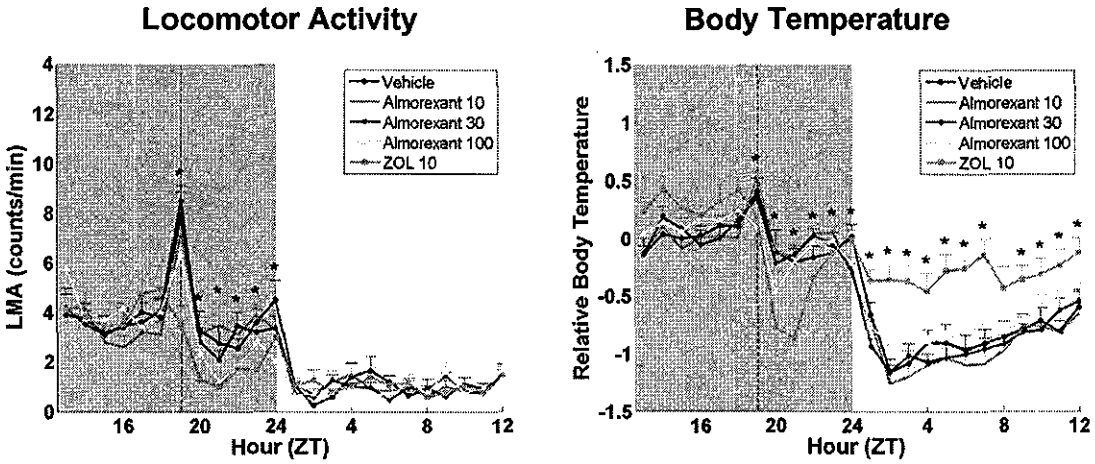


Figure 4. Average hourly LMA and relative T_{core} for 6 h prior to and 18 h after injections. Shaded area indicates dark phase; the dashed line in each panel indicates the first h following dosing. **Left:** The average hourly LMA for 3 concentrations of almorexant vs. ZOL and vehicle. ANOVA for ZT19-ZT24 is significant for treatment (F=7.31, P=0.00036) and for treatment by time (F=2.38, P=0.0018). For treatment by time: At ZT19, ZOL<all other conditions. At ZT20, ZOL<almorexant at 10 mg/kg and vehicle. At ZT21, ZOL<almorexant at 10 and 30 mg/kg and vehicle. At ZT22, ZOL<almorexant at 30 mg/kg and vehicle. At ZT23, ZOL<almorexant at 10

and 30 mg/kg and vehicle. At ZT24, almorexant at 100 mg/kg and ZOL<vehicle. **Right:** The average hourly T_{core} for 3 concentrations of almorexant vs. ZOL and vehicle. ANOVA for ZT19-ZT24 is significant for treatment (F=7.55, P=0.00029) and for treatment by time (F=3.97, P<0.00001). ANOVA for ZT1-ZT6 is significant for treatment (F=15.75, P<0.00001) and for treatment by time (F=1.95, P=0.0134). ANOVA for ZT7-ZT12 is significant for treatment (F=7.92, P=0.00021) and for treatment by time (F=1.90, P=0.0167). For treatment by time: At ZT19, almorexant at 100 mg/kg<vehicle. At ZT20, ZOL<almorexant at 30 mg/kg and vehicle. At ZT21, ZOL<all other conditions. At ZT22, vehicle<almorexant at 30 mg/kg. At ZT23, ZOL<almorexant at 10 mg/kg. At ZT24, almorexant at 10 mg/kg<vehicle. At ZT1, almorexant at all concentrations<ZOL. At ZT2, all other conditions<ZOL. At ZT3, all other conditions<ZOL. At ZT4, all other conditions<ZOL. At ZT5, all other conditions<ZOL. At ZT6, all other conditions<ZOL. At ZT7, all other conditions<ZOL. At ZT8, all other conditions<ZOL. At ZT9, all other conditions<ZOL. At ZT10, almorexant at 10 and 30 mg/kg and vehicle<ZOL. At ZT11, almorexant at 10 mg/kg and vehicle<ZOL; vehicle<almorexant at 100 mg/kg. At ZT12, almorexant at 10 and 30 mg/kg and vehicle<ZOL.

Task 3. *Test the hypothesis that the Hcrt antagonist ALM induces sleep by selectively disfacilitating the activity of the histaminergic, serotonergic, noradrenergic and cholinergic wake-promoting systems whereas the BzRA ZOL causes a generalized inhibition of the brain.*
3a. Double-label immunohistochemistry with Fos and phenotypic markers (months 1 to 12).
3b. Assessment of hypnotic efficacy in saporin-lesioned rats (months 13 to 24).
3c. Assessment of hypnotic efficacy in transgenic mice (months 25 to 36).

Progress: In the absence of tissue from ALM- and ZOL-treated animals, there has been little experimental progress on this task in Year 1 and little expenditure of funds. The primary effort to date has been to order the appropriate antisera for these experiments and to initiate establishment of the immunohistochemical assays. We anticipate receiving the first group of 24 animals (8 ALM-treated, 8 ZOL-treated and 8 vehicle controls) by the end of August and work on this Task will accelerate at that time.

With the approval of Ms. Jennifer Shankle of MEDCOMM USAMRAA received on 10 Jun 2010, we re-budgeted some of the Year 1 funds to allow an upgrade of our existing Neurolucida and StereoInvestigator software from Microbrightfield, Inc. The PC and software upgrade was received in our laboratory on 26 Jul 2010. At the time of this writing, we are awaiting installation by a service representative. This data analysis package is necessary to conduct the cell counts necessary for quantification of the studies to be executed in Tasks 3 and 4a.

Task 4. *Test the hypothesis that ALM, but not ZOL, induces sleep by facilitating the mechanisms that underlie the transition to normal sleep.*
4a. Effects of ALM and ZOL on sleep-active brain areas (months 1 to 12).
4b. BF adenosine (ADO) release in response to oral ALM and ZOL (months 1 to 24).
4c. BF adenosine (ADO) release in response to ALM and ZOL by dialysis (months 25 to 48).

Progress: As in Task 3, progress on experiments has been limited due to the construction issues described above. However, there has been tremendous progress on the infrastructure to support

Task 4. As indicated above, renovations are ongoing within the Animal Facility to construct a state-of-the-art 535 s.f. laboratory for EEG/EMG, behavioral performance and microdialysis sample collection.

In addition, shortly after funding of DR080789P1 (USAMRAA Grant W81XWH-09-2-0080), we were assigned a 985 square foot wet laboratory in which to establish an Analytical Neurochemistry Facility. LB212 has 6 bays and contains 6 fume hoods. To equip this laboratory, we relocated our one ESA CoulChem III High Performance Liquid Chromatograph (HPLC) used to detect dopamine and its metabolites to LB212 and, using SRI internal funds, we were able to acquire several other used ESA Coul Arrays from Roche Palo Alto. These additional machines enable us to measure acetylcholine, norepinephrine and its metabolites, and serotonin and its metabolites, as described in our 10 Feb 2010 email to Dr. del Carmen. In addition, an HPLC for the detection of adenosine, which had been requested in the original budget for DR080789P1 (USAMRAA Grant W81XWH-09-2-0080), was delivered on 19 Jul 2010. Lastly, on 10 Jun 2010, we received approval from Ms. Jennifer Shankle of MEDCOMM USAMRAA to re-budget some of the Year 1 funds to allow purchase of a 6th HPLC for determination of GABA, glutamate, glycine and other amino acids. We expect delivery of this machine by the end of this week. Thus, we will soon have capabilities well beyond the scope of the adenosine measurements proposed as Task 4b and 4c in the SOW.

We report here on progress to establish the functionality of these machines. Our ESA-Dionex service representative performed a full system reinstallation of all hardware equipment and software, and validated communication and automation capabilities for three ESA Coul Arrays. Service reports for each HPLC system are attached in Appendix 3. We then set up the three HPLCs for electrochemical detection, each to be optimized for specific neurotransmitter capabilities (System 1: norepinephrine, epinephrine, and dopamine; System 2: serotonin; System 3: acetylcholine). The last task was to validate internal standards tested specifically for each system to determine the lower limit of detection (i.e., what is the lowest level of neurotransmitter amount that can be measured *in vivo*).

For the data presented in Figure 5, individual samples were automatically injected by an autosampler into the HPLC/EC system (ESA-Dionex, Chelmsford, MA) for the generation of an external standard curve for norepinephrine (NE), epinephrine (Epi), and dopamine (DA). All neurotransmitter concentrations were made up as stock solutions and were dissolved in oxalic acid (1 mM, pH 3.6), and serially diluted to their final concentrations in 1 mM oxalic acid to preserve the stability of the samples. The mobile phase consisted of 150 mM Na_2HPO_4 (pH = 5.6), 3 mM sodium dodecyl sulfate, 50 mM EDTA, 10% methanol, and 15% acetylnitrile. NE, Epi, and DA were carried through with mobile phase, separated through an analytical MD-150x3.2-mm reversed phase column from ESA-Dionex, and oxidized/reduced using a Coul Array detector from ESA, Inc. Two electrodes were used, a reduction analytical electrode (E1, -0.1 V), and an oxidation analytical electrode (E2, 0.25 V). The area under the curve of each peak was measured using CoulArray Data Station 3.0 software (ESA, Inc.). Figure 5A shows the plot generated by the software and corresponding external standard data points for each neurotransmitter type: NE (blue circles), Epi (red circles), and DA (green circles). Individual samples of known concentrations were run in duplicate and averaged peak areas were integrated into a linear fit model to provide the goodness of fit (r value) for each neurotransmitter (NE, r=0.9995; Epi, r=0.9999, and DA, r=1.000). Figure 5B shows the calibration levels that were generated for each neurotransmitter and their corresponding averaged peak areas for each concentration. The lowest amounts of neurotransmitters detected using this calibration curve for

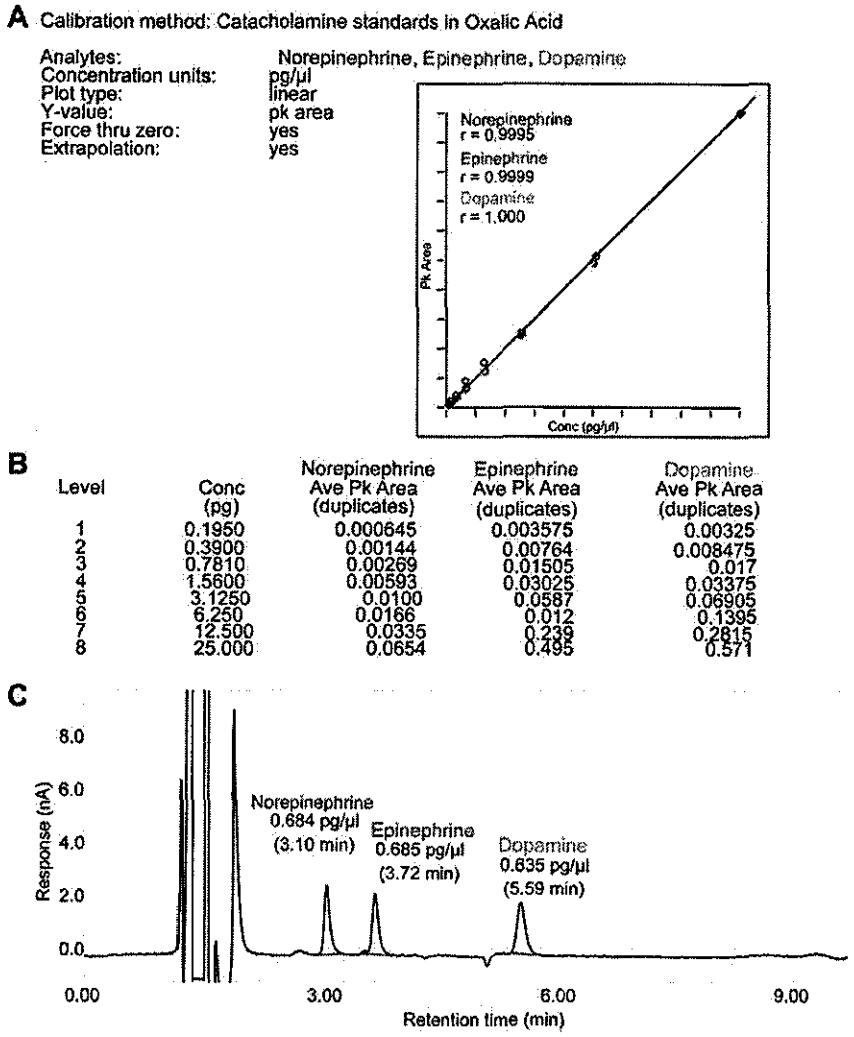


Figure 5. External standards, calibration, and HPLC/EC detection of norepinephrine (NE), epinephrine (Epi), and dopamine (DA).

NE, Epi, and DA were 200 fg, 200 fg, and 500 fg, respectively. Figure 5C depicts a chromatograph showing individual peaks for NE, Epi, and DA and their respective concentrations and retention times.

For the data presented in Figure 6, individual samples were injected by automation into the HPLC/EC system (ESA-Dionex) for the generation of an external standard curve for serotonin (5-HT). Serotonin concentrations was made up as a stock solution and was dissolved in oxalic acid (1 mM, pH 3.6), and serially diluted to final concentrations in 1 mM oxalic acid to preserve the stability of the samples. The mobile phase consisted of 150 mM Na_2HPO_4 (pH = 5.6), 3 mM sodium dodecyl sulfate, 50 mM EDTA, 10% methanol, and 15% acetylnitrile. 5-HT was carried through with mobile phase, separated through an analytical MD-150x3.2-mm reversed phase column from ESA-Dionex, and oxidized/reduced using a Coul Array detector from ESA, Inc. Two electrodes were used, a reduction analytical electrode (E1, -0.1 V), and an oxidation analytical electrode (E2, 0.25 V). The area under the curve of each peak was measured using CoulArray Data Station 3.0 software (ESA, Inc.). Figure 6A plots the peak area detected against the corresponding 5-HT external standards (blue circles). Individual samples of known concentrations were run in duplicate and averaged peak areas were integrated into a linear fit model to provide the goodness of fit (r value) for 5-HT (r=0.9968). Figure 6B shows the calibration levels that were generated for 5-HT and the corresponding averaged peak areas for each calibrated amount of 5-HT concentration. The lowest amount of neurotransmitter detection using this calibration curve for serotonin was 1 pg on column. Figure 6C presents a chromatograph showing an individual serotonin peak with its respective concentration and retention time.

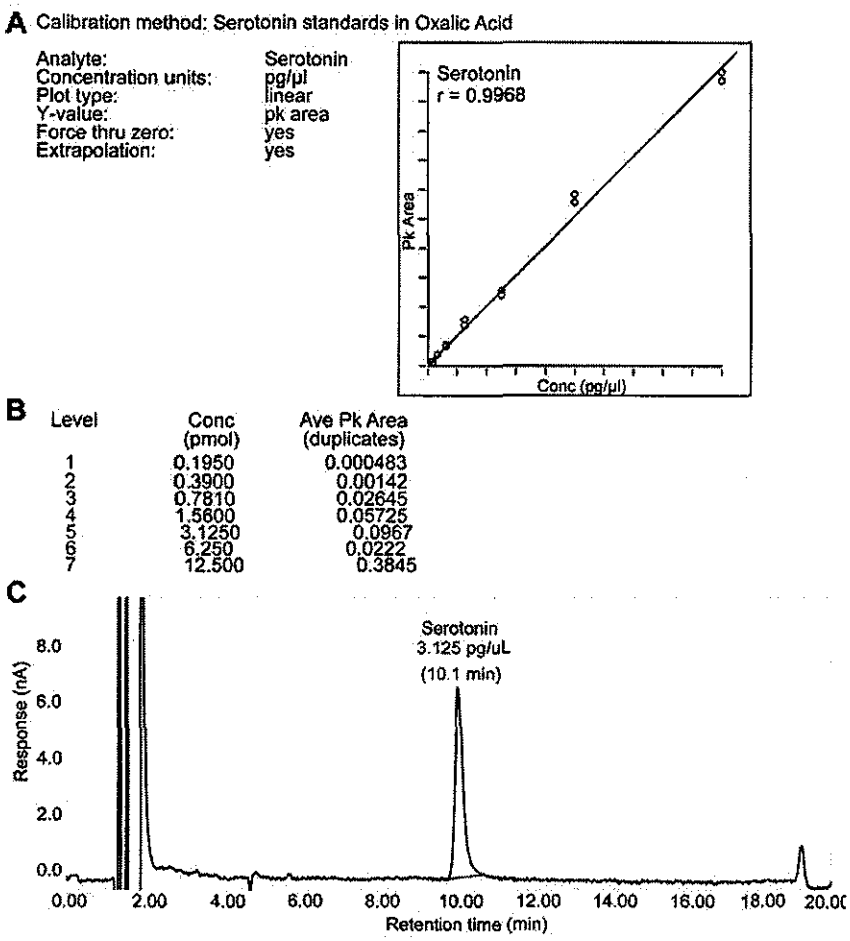


Figure 6. External standards, calibration, and HPLC/EC detection of serotonin (5-HT).

For the data presented in Figure 7, individual samples were automatically injected via an autosampler into the HPLC/EC system (ESA-Dionex) to generate an external standard curve for acetylcholine (ACh). ACh was made up as a stock solution and was dissolved in oxalic acid (1 mM, pH 3.6), and serially diluted to final concentrations in 1 mM oxalic acid to preserve the stability of the samples. The mobile phase consisted of 100 mM Na_2HPO_4 2 mM 1-octanesulfonic acid, and adjusted to pH = 8.0 with phosphoric acid. This HPLC method uses a polymeric stationary phase to resolve choline (Ch) from ACh and is attached to an ACH-250x3.0-mm column. Analytes are then converted to hydrogen peroxide (H_2O_2) by a solid-phase reactor (containing immobilized choline oxidase and acetylcholinesterase enzymes). An additional enzyme reactor is attached to the column to eliminate the choline peak and avoid interference with ACh retention time. The H_2O_2 is detected amperometrically and quantified on a platinum (Pt) working electrode set to +300 mV with a solid-state palladium reference electrode. The area under the curve of each peak was measured using CoulArray Data Station 3.0 software (ESA, Inc.). Figure 7A plots peak areas detected against external ACh standards (blue circles). Individual samples of known concentrations were run in duplicate and averaged peak areas were integrated into a linear fit model to provide the goodness of fit (r value) for ACh (r=0.9980). Figure 7B lists the calibration levels that were generated for ACh and the corresponding averaged peak areas for each calibrated amount of ACh concentration. The lowest amount of neurotransmitter detection using this calibration curve for ACh was 200 fmol. Figure 7C depicts a chromatograph showing an individual ACh peak with its respective concentration and retention time.

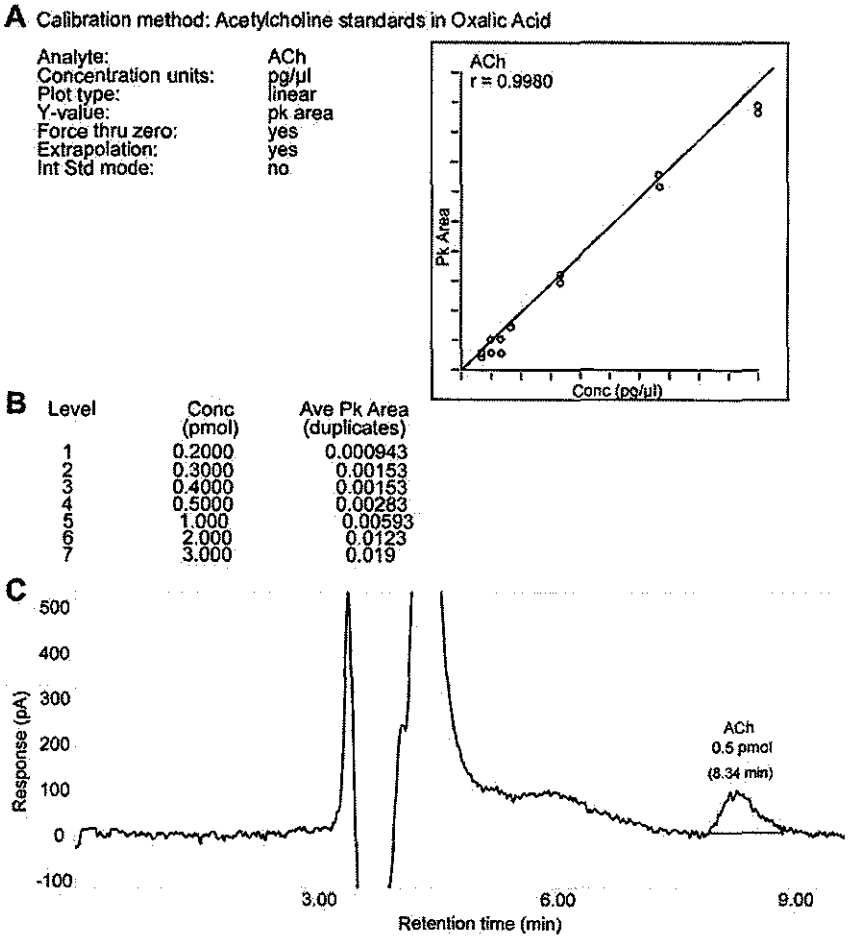


Figure 7. External standards, calibration, and HPLC/EC detection of acetylcholine (ACh).

Task 5: *Test the hypothesis that neural gene expression that occurs ALM-induced sleep more closely resembles that of spontaneous sleep than does ZOL-induced sleep.*
5a. Comparison of ALM and ZOL effects on expression of plasticity-related genes (months 37 to 48).
5b. Comparison of ALM and ZOL effects on brain gene expression in comparison to spontaneous sleep (months 37 to 48).

Progress: None anticipated prior to Year 3.

KEY RESEARCH ACCOMPLISHMENTS

• Obtaining approval and commitment from SRI International to construct a new 535 s.f. laboratory suite within the Animal Facility to support the *in vivo* portion of this research program (construction initiated 19 May 2010; expected occupancy 3 Aug 2010).

• Set up of the water maze and video tracking system and initiation of data collection in a temporary location until construction of above-mentioned laboratory suite is completed

• Establishment of a 985 s.f. Analytical Neurochemistry Facility in LB212 to support this research program containing:

- 1 ESA CoulChem HPLC for analysis of dopamine and its metabolites relocated to LB212.
- 3 ESA Coul Array HPLCs for analysis of acetylcholine, norepinephrine and serotonin purchased from Roche Palo Alto on internal SRI funds; setup of machines supported by rebudgeting of current grant.
- 1 HPLC for analysis of adenosine received on 19 Jul 2010; awaiting installation.
- 1 HPLC for analysis of GABA, glutamate, glycine and other amino acids ordered on 14 Jun 2010; delivery expected this week.

• Full system reinstallation of all hardware equipment and software, and validated communication and automation capabilities for three ESA Coul Array HPLCs.

• Establishment of limits of detection for 4 of the ESA Coul Array HPLCs (Figs. 5-7).

• Determination of the effect of 3 doses of ALM vs. ZOL on sleep/wake and other physiological parameters in the Sprague-Dawley rat (Figs. 1-4)

• Upgrade of our existing Neurolucida and StereoInvestigator software from Microbrightfield, Inc. to facilitate cell counts necessary for quantification of the studies to be executed in Tasks 3 and 4a.

REPORTABLE OUTCOMES

Manuscript in preparation:

Morairty SR, F.G. Revel, P. Malherbe, J-L. Moreau, K. Silveira, D. Valladao, J.G. Wettstein, T.S. Kilduff, E. Borroni. Dual hypocretin receptor antagonism is more effective for sleep promotion than antagonism of either receptor alone.

CONCLUSION

Preclinical data indicate that animals treated with ALM are easily aroused from sleep and are free of ataxia and other behavioral impairments. If this observation is confirmed in humans, it would have enormous implications for the management of disturbed sleep in both military and civilian populations. Our research in this area has just commenced in Year 1 of this project but we expect to be able to address the validity of these claims and the mechanisms that may underlie them in the near future.

REFERENCES

Morairty, S. L. Hedley, J. Flores, R. Martin and T. S. Kilduff (2008). Selective $5HT_{2A}$ and $5HT_6$ receptor antagonists promote sleep in rats. *Sleep* 31:34-44.

APPENDICES

Appendix 1. Schematic floorplan of new EEG/EMG, Performance and Microdialysis Laboratory in LW103/105.

Appendix 2. Certificate of Analysis for synthesis of Almorexant by SRI International Medicinal Chemistry Laboratory.

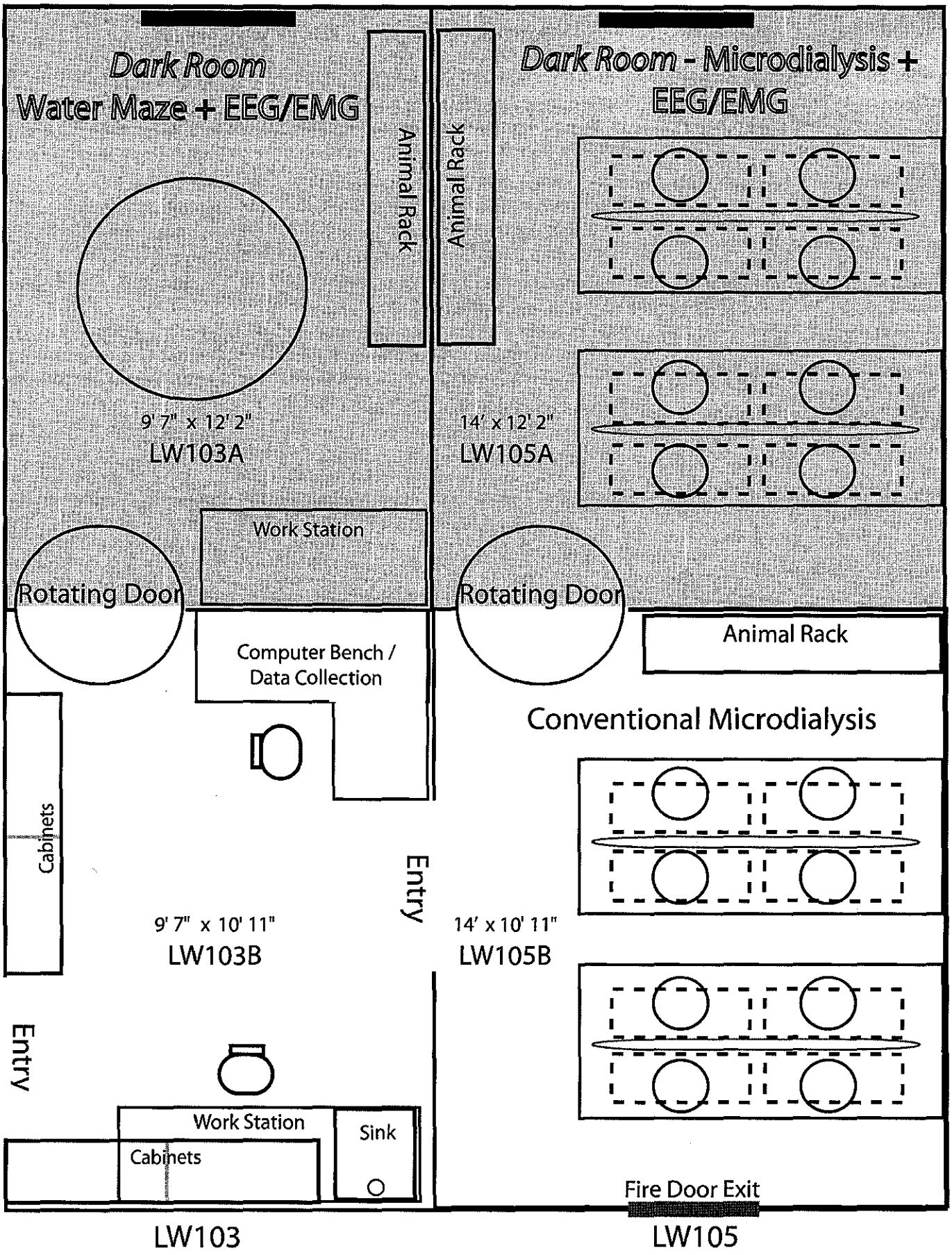
Appendix 3. Service report for the reinstallation and calibration of HPLC/EC system #1 (detection of norepinephrine, epinephrine, and dopamine) by ESA-Dionex, Inc.

Appendix 4. Service report for the reinstallation and calibration of HPLC/EC system system #2 (detection of serotonin) by ESA-Dionex, Inc.

Appendix 5. Service report for the reinstallation and calibration of HPLC/EC system #3 (detection of acetylcholine) by ESA-Dionex, Inc.

Appendix 1

Schematic floorplan of new EEG/EMG,
Performance and Microdialysis Laboratory in LW103/105.


Dark Room
Water Maze + EEG/EMG
Animal Rack
Animal Rack
Dark Room - Microdialysis +
EEG/EMG
9' 7" x 12' 2"
LW103A
14' x 12' 2"
LW105A
Work Station
Rotating Door
Rotating Door
Animal Rack
Computer Bench /
Data Collection
Conventional Microdialysis
Cabinets
Entry
9' 7" x 10' 11"
LW103B
14' x 10' 11"
LW105B
Entry
Work Station
Sink
Cabinets
Fire Door Exit
LW103
LW105

Appendix 2

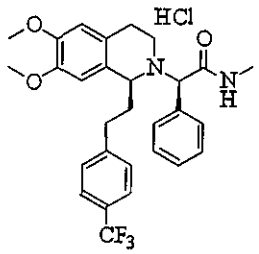
Certificate of Analysis for synthesis of Almorexant
by SRI International Medicinal Chemistry Laboratory.

Certificate of Analysis

To: Dr. Thomas Kilduff	**Date: 3/31/2010**
	Contractor: SRI International 333 Ravenswood Avenue Menlo Park, CA 94025
Almorexant (codenamed ACT-078573)	**P.I.:** Dr. Ling Jong
Attn: Stephen Morairty	**Project No:** P 19160.102

Chemical Name: (R)-2-((S)-6,7-dimethoxy-1-(4-(trifluoromethyl)phenethyl)-3,4-dihydroisoquinolin-2(1H)-yl)-N-methyl-2-phenylacetamide hydrochloride

Structure:



Lot #: GL-S14124-24-F1		**ELEMENTAL**	**Calculated**	**Found**
Formula: C29H32ClF3N2O3		**C**	63.44	63.41
Molecular Weight: 549.02		**H**	5.87	5.79
Date Synthesized: 03/09/2010		**N**	5.10	5.07
Purity: > 99%	**MP:** 205-206			
Analyst: Adria Lombardo			Amount: 26.05 g	
Reviewer: Dr. Gaoquan Li			Store in 12 oz brown bottle	

NMR: ^{1}H (300 MHz) ($CDCl_3$) δ 12.61 (br.s, 1 H), 9.53 (br.s, 1 H), 7.62 (br.s, 2 H), 7.48-7.30 (m, 5 H), 7.09 (d, J = 7.6 Hz, 2 H), 6.68 (s, 1 H), 5.73 (s, 1 H), 4.54 (d, J = 10.4 Hz, 1 H), 4.01-3.86 (m, 4 H), 3.86-3.76 (m, 2 H), 3.67 (s, 3 H), 3.34-3.22 (m, 1 H), 3.17-3.02 (m, 2 H), 2.89 (d, J = 4.4 Hz, 3 H), 2.86-2.73 (m, 2 H), 2.03-1.91 (m, 1 H)

MS: ESI+: 513.1, ESI-: 511.2 (non-salt Almorexant)

UV: (Methanol) λ_{max} 286.1 nm (ε 4,099); 204.1 (ε 55,555)

FTIR: (Film) 3177.7, 3043.6, 2932.8, 2581.4, 1681.4, 1522.9, 1461.5, 1329.2, 1264.4, 1232.7, 1160.9, 1110.4, 1071.4, 1019.0, 743.9, 697.7 cm^{-1}

TLC: Analtech silica gel plates; 60% ethyl acetate/40% hexane R_f = 0.25 (non-salt Almorexant)

HPLC: Phenomenex RP-C18; flow 1 mL/min; detection 284.0 nm; solvent 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B), 10%-90% B over 15 min; retention time 12.54 min; purity > 99 %

Appendix 3

Service report for the reinstallation and calibration of HPLC/EC system #1 (detection of norepinephrine, epinephrine, and dopamine) by ESA-Dionex, Inc.

ESA Biosciences
22 Alpha Road
Chelmsford, MA 01824
Telephone: (800) 275-0102
Fax: (978) 250-7092

Service Report

Service Request #	538304	Engineer	Ralf Janssen
Type of Service	Installation	Date	February 26, 2010

Customer Information

Company	SRI International
Address	333 Ravenswood Ave
Address	
Address	
City	Menlo Park
State	CA
Zip	94025

Instrument Information

		Model	Serial Number
Detector	CoulArray	5600	CA-856
Autosampler	ESA	542	60100
Pump 1	ESA	582	S20104351055
Pump 2			
Other			

Contact	Jacqueline Vasquez-DeRose	Customer Number	
Phone Number	650-859-4794	Purchase Order	98-000219
Fax Number		Sales Order	204584
E-Mail Address	jacqueline.vasquez@sri.com	Work Order	

DESCRIPTION OF SERVICE TO BE PERFORMED

Reinstall Coularray, Autosampler, Pump and computer. Install Coularray for Windows v3.1

DESCRIPTION OF WORK PERFORMED

Reinstallation of Coularray, Autosampler, Pump and computer completed. Installed Coularray for Windows v3.1

Did the work performed on this request require that a product complaint be generated

If yes, Product complaint #

Service Request # 538304

PARTS REQUIRED

QUANTITY	PART NUMBER	DESCRIPTION	INVENTORY	LIST PRICE	DISCOUNT	TOTAL PRICE
1	70-4003	CoulArray for Windows v3.1	SVS RALF	2,860.00		2,860.00

						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
			PARTS SUBTOTAL			2,860.00
			DISCOUNT ON SUBTOTAL			2,860.00
TRAVEL AND LABOR						
1	70-7645	ESA HPLC Installation Service		1985		1,985.00
1	70-7646	ESA Software Installation Service		995		995.00
1	70-7641	Zone Travel Charge		400		400.00
						-
				TOTAL	$	6,240.00

SUGGESTIONS / COMMENTS

autosampler cooling tray liner deteriorating: recommend replacing.

Service Representative

Ralf Janssen — *PLEASE PRINT*

SIGNATURE — *DATE* 2/26/2010

Customer

Jacqueline Vazquez-DeRose — *PLEASE PRINT*

SIGNATURE — *DATE* 2/24/10

Appendix 4

Service report for the reinstallation and calibration of
HPLC/EC system system #2 (detection of serotonin) by ESA-Dionex, Inc.

ESA Biosciences
22 Alpha Road
Chelmsford, MA 01824
Telephone: (800) 275-0102
Fax: (978) 250-7092

A Dionex Company

Service Report

Service Request #	538306	Engineer	Ralf Janssen
Type of Service	Installation	Date	February 26, 2010

Customer Information

Company	SRI International
Address	333 Ravenswood Ave
Address	
Address	
City	Menlo Park
State	CA
Zip	94025

Instrument Information

		Model	Serial Number
Detector	CoulArray	5600	CA-909
Autosampler	ESA	542	50358
Pump 1	ESA	582	S20104390994
Pump 2			
Other			

Contact	Jacqueline Vazquez-DeRose+C106	Customer Number	
Phone Number	650-859-4794	Purchase Order	98-000219
Fax Number		Sales Order	204584
E-Mail Address	jacqueline.vasquez@sri.com	Work Order	

DESCRIPTION OF SERVICE TO BE PERFORMED

Reinstall Coularray, Autosampler, Pump and computer. Install Coularray for Windows v3.1

DESCRIPTION OF WORK PERFORMED

Reinstallation of Coularray, Autosampler, Pump and computer completed. Installed Coularray for Windows v3.1

Did the work performed on this request require that a product complaint be generated

If yes, Product complaint #

Service Request # 538306

PARTS REQUIRED

QUANTITY	PART NUMBER	DESCRIPTION	INVENTORY	LIST PRICE	DISCOUNT	TOTAL PRICE
1	70-4003	CoulArray for Windows v3.1	SVS RALF	2,860.00		2,860.00
						-

						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
				PARTS SUBTOTAL		2,860.00
				DISCOUNT ON SUBTOTAL		2,860.00
TRAVEL AND LABOR						
1	70-7645	ESA HPLC Installation Service		1985		1,985.00
						-
						-
						-
				TOTAL	$	4,845.00

SUGGESTIONS / COMMENTS

Service Representative

Ralf Janssen
PLEASE PRINT

SIGNATURE

2/26/2010
DATE

Customer

Jacqueline Vazquez-DeRose
PLEASE PRINT

SIGNATURE

2/26/10
DATE

Appendix 5

Service report for the reinstallation and calibration of HPLC/EC system #3 (detection of acetylcholine) by ESA-Dionex, Inc.

ESA Biosciences
22 Alpha Road
Chelmsford, MA 01824
Telephone: (800) 275-0102
Fax: (978) 250-7092

A Dionex Company

Service Report

Service Request #	538308	Engineer	Ralf Janssen
Type of Service	Installation	Date	February 26, 2010

Customer Information

Company	SRI International
Address	333 Ravenswood Ave
Address	
Address	
City	Menlo Park
State	CA
Zip	94025

Instrument Information

		Model	Serial Number
Detector	CoulArray	5600	CA-935
Autosampler	ESA	542	40266
Pump 1	ESA	582	S20104250866
Pump 2			
Other			

Contact	Jacqueline Vazquez-DeRose	Customer Number	
Phone Number	650-859-4794	Purchase Order	98-000219
Fax Number		Sales Order	204584
E-Mail Address	jacqueline.vasquez@sri.com	Work Order	

DESCRIPTION OF SERVICE TO BE PERFORMED

Reinstall Coularray, Autosampler, Pump and computer. Install Coularray for Windows v3.1

DESCRIPTION OF WORK PERFORMED

Reinstallation of Coularray, Autosampler, Pump and computer completed. Installed Coularray for Windows v3.1

Did the work performed on this request require that a product complaint be generated

If yes, Product complaint #

Service Request # 538308

PARTS REQUIRED

QUANTITY	PART NUMBER	DESCRIPTION	INVENTORY	LIST PRICE	DISCOUNT	TOTAL PRICE
1	70-4003	CoulArray for Windows v3.1	SVS RALF	2,860.00		2,860.00
						-
FSR SIDE 1					FORM -024 REV.-C	

						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
						-
				PARTS SUBTOTAL		2,860.00
				DISCOUNT ON SUBTOTAL		2,860.00
TRAVEL AND LABOR						
1	70-7645	ESA HPLC Installation Service		1985		1,985.00
						-
						-
						-
				TOTAL	$	4,845.00

SUGGESTIONS / COMMENTS

Service Representative

Ralf Janssen — *PLEASE PRINT*

SIGNATURE 2/26/2010 — *DATE*

Customer

Jacqueline Vazquez-DeRose — *PLEASE PRINT*

SIGNATURE 2/26/10 — *DATE*

Appendix 3: IND Authorization Letter

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

IND 110,059

IND ACKNOWLEDGEMENT

Thomas Neylan, M.D.
Department of Veterans Affairs Medical Center
4150 Clement St. (116P)
San Francisco, Ca 94121

Dear Dr. Neylan:

We acknowledge receipt of your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act (FDCA). Please note the following identifying data:

IND NUMBER ASSIGNED: **110,059**

SPONSOR: **Thomas Neylan, M.D.**

PRODUCT NAME(S): **Almorexant 100 mg Tablets**

DATE OF SUBMISSION: **September 17, 2010**

DATE OF RECEIPT: **September 21, 2010**

You may not initiate studies in humans until 30 days after the date of receipt shown above unless we notify you sooner that you may proceed. If, on or before **October 21, 2010**, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies, we will immediately notify you verbally or in writing that (1) clinical studies may not be initiated under this IND ("clinical hold") or (2) certain restrictions apply to clinical studies under this IND ("partial clinical hold"). If we place your human studies on clinical hold, you will be notified in writing of the reasons and the information necessary to correct the deficiencies. In the event of such notification, you must not initiate or you must restrict such studies until you have submitted information to correct the deficiencies, and we have subsequently notified you that the information you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND. However, if the drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

As sponsor of this IND, you are responsible for compliance with the FDCA (21 U.S.C. §§ 301 et. seq.) as well as the implementing regulations [Title 21 of the Code of Federal Regulations (CFR)]. A searchable version of these regulations is available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm. Your responsibilities include:

- Reporting any unexpected fatal or life-threatening adverse experiences associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)];

- Reporting any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, in writing to this Division and to all investigators within 15 calendar days after initial receipt of this information [21 CFR 312.32(c)(1)]; and

- Submitting annual progress reports within 60 days of the anniversary of the date that the IND went into effect (the date clinical studies were permitted to begin) [21 CFR 312.33].

You are also responsible for complying with the applicable provisions of sections 402(i) and (j) of the Public Health Service Act (PHS Act) (42 USC §§ 282 (i) and (j)), which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No, 110-85, 121 Stat. 904).

We remind you that, under 21 CFR 312.8(a)(3), you may not charge for this investigational drug without prior written authorization from FDA.

GOOD LABORATORY PRACTICE

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58). If such studies have not been conducted in compliance with these regulations, provide a statement describing in detail all differences between the practices used and those required in the regulations.

ENVIRONMENTAL ASSESSMENT

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. Information on environmental assessments is available in the guidance "Environmental Assessment of Human Drugs and Biologics." This document is available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070561.pdf.

PEDIATRIC ASSESSMENTS

The Pediatric Research Equity Act of 2003 (PREA) (Public Law 108-155) addresses drug and biological product development for pediatric uses. All sponsors have obligations to study pediatric populations as outlined in PREA. Under PREA, all applications submitted under section 505 of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. 355) or section 351 of the Public Health Service Act (PHS Act) (42 U.S.C. 262) for a new active ingredient, new indication, new dosage form, new dosing regimen, or new route of administration are to contain a pediatric assessment (pediatric clinical data) unless the sponsor has obtained a waiver or deferral from FDA (21 U.S.C. 355c). A draft guidance on the implementation of PREA was issued by FDA in September 2005. As stated in that document, FDA encourages the submission of pediatric development plans to FDA as early as possible in the product development process to increase understanding of immunogenicity, dosing, and safety information, etc. in the pediatric population.

ABUSE POTENTIAL ASSESSMENT

Drugs that affect the central nervous system, are chemically or pharmacologically similar to other drugs with known abuse potential, or produce psychoactive effects such as mood or cognitive changes (e.g., euphoria, hallucinations) need to be evaluated for their an abuse potential and a proposal for scheduling will be required at the time of the NDA submission [21 CFR 314.50(d)(5)(vii)]. For information on the abuse potential evaluation and information required at the time of your NDA submission, see the draft guidance for industry, "Guidance for Industry Assessment of Abuse Potential of Drugs", available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM198650.pdf.

SUBMISSION REQUIREMENTS

Cite the IND number listed above at the top of the first page of any communications concerning this application. Each submission to this IND must be provided in triplicate (original plus two copies). Please include three originals of all illustrations that do not reproduce well. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Neurology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

All regulatory documents submitted in paper should be three-hole punched on the left side of the page and bound. The left margin should be at least three-fourths of an inch to assure text is not obscured in the fastened area. Standard paper size (8-1/2 by 11 inches) should be used; however, it may occasionally be necessary to use individual pages larger than standard paper size. Non-standard, large pages should be folded and mounted to allow the page to be opened for review without disassembling the jacket and refolded without damage when the volume is

shelved. Shipping unbound documents may result in the loss of portions of the submission or an unnecessary delay in processing which could have an adverse impact on the review of the submission. For additional information, see http://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/DrugMasterFilesDMFs/ucm073080.htm.

If you have any questions, call me, at (301) 796-1123.

Sincerely,

{See appended electronic signature page}

Cathleen Michaloski, BSN, MPH
Sr. Regulatory Project Manager
Division of Neurology Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

CATHLEEN B MICHALOSKI
09/27/2010

Appendix 4: Agenda for Annual Meeting between San Francisco (human site) and SRI International (animal site)

Hypocretin Joint Meeting – SRI International and NCIRE
Friday, June 4, 2010
12:00 pm - 5:00 pm
San Francisco VA Medical Center, Building 8, Room 313

PRESENTERS

NCIRE Team:	
Thomas Neylan, M.D.	Director, PTSD Research Program, San Francisco VA Medical Center and Professor, UCSF Department of Psychiatry
Steven Batki, M.D.	Director, Addiction Research Program, San Francisco VA Medical Center and Professor, UCSF Department of Psychiatry
SRI International Team:	
Thomas Kilduff, Ph.D.	Senior Director, Center for Neuroscience
Stephen Morairty, Ph.D.	Associate Director, Center for Neuroscience
Jacqueline Vazquez, Ph.D.	Scientist, Center for Neuroscience
Larry Toll, Ph.D.	Senior Director, Neuropharmacology Program, Center for Neuroscience
Tanya Wallace, Ph.D.	Director, Translational Neuroscience Program, Center for Neuroscience

AGENDA

TIME	TOPIC	PRESENTER
12:00pm	Lunch, Introductions	All
12:30 – 12:45	Overview of Work to Date	Thomas Neylan, Thomas Kilduff
12:50 – 1:20	Hypothalamic and Cortical Involvement in Sleep and Wakefulness	Thomas Kilduff
1:20 – 1:50	Hypocretin Receptor Antagonism and Sleep Promotion: Is 2 > 1+2?	Stephen Morairty
1:50 – 2:00	Break	All
2:00 – 2:20	Microdialysis Research Capabilities at SRI International	Jacqueline Vazquez
2:20 – 2:40	Translating Preclinical Research into the Clinic Using Higher Order Species	Tanya Wallace
2:40 – 3:00	Nociceptin and NOP Agonists as Drug Abuse Medications	Larry Toll
3:00 – 3:20	NIDA and NIAAA Interest in the Application of Hypocretin in Substance Abuse Disorders	Steven Batki
3:20 – 3:30	Break	
3:30 – 3:50	Proposed Neurocognitive Battery Refinements in Human Study	Thomas Neylan
3:50 – 4:00	Preview of Upcoming CRF Antagonist Trial	Thomas Neylan
4:00 – 4:45	Planning Ahead: New Directions	All
4:45 – 5:00	Wrap-Up	All